

**Effect of temperature on the  
physiology of two  
exotic frogs: possible causes of  
distribution**

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of Master of Zoology in Physiology

by Leon Akila Glynne Allen

School of Biological Sciences

University of Canterbury

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## Abstract:

Two Australian frogs were introduced to New Zealand over 100 years ago. Since their introduction they have become widespread and well established with *Litoria ewingii* being more prevalent in alpine and cooler areas of New Zealand, while *Litoria raniformis* is found in more temperate coastal areas. Very little physiological data exists for these frogs and aside from their distribution not much is known about them in New Zealand. Thus the effects of thermal acclimation and temperature change on respiration and locomotion were examined in these two exotic frogs. The more terrestrial and alpine dwelling *L. ewingii* was found to be able to thermally acclimate its respiration performance, where respiration was highest when acclimation temperature matched test temperature. It was also able to thermally acclimate its locomotory performance, jumping significantly further at lower temperatures, however, acclimation to high temperatures impacted its jump performance at cold temperatures. The frog *L. raniformis* was found to only be able to thermally acclimate its respiration and locomotion to high temperatures, as performance at low temperatures was often poor. The data shows that *L. ewingii* is a cold temperate frog rather than a warm habitat frog, while *L. raniformis* is an animal adapted to warm temperatures. From this we can begin to see the effect that temperature has on the physiology of these two exotic frogs and the major role that temperature may be playing in driving the differences seen in the distribution of these two species in New Zealand.

# 1. General Introduction

## 1.1 Introduction:

Two Australian frogs, *Litoria raniformis* (Southern Bell Frog) and *Litoria ewingii* (Brown Tree Frog), were introduced into New Zealand in the late 1800s. Both frogs are native to Australia and are found primarily in South Australia, New South Wales, and Tasmania (Gartside, 1982; Pyke, 2002). Since their introduction they have become well established and widespread (Cree, 1984), though both species still exhibit similar biology to populations in Australia (Cousineau, 1990; Voros *et al*, 2008). A major difference between these two closely related species is the habitat they occupy and their breeding biology. *L. raniformis* has generally been reported to occur in or around water, such as ponds, and slow moving or still streams, however they have also been found to occur in lakes, marshes, swamps, wetlands, and even artificial areas, such as farm dams, irrigation races and garden ponds (Pyke, 2002). Most breeding activity in *L. raniformis* occurs in spring and summer months, with calling by males generally heard during this time (Cree, 1984). In contrast, *L. ewingii* is more terrestrial and can be found at large distances from water. It has been shown to inhabit areas such as grasslands, alpine forests, and gardens (Cree, 1984). *L. ewingii* is a highly tolerant species; this is exemplified by its ability to tolerate freezing (Bazin *et al*, 2007), as well as spawning in naturally acidic waters (Cousineau, 1990).

## 1.2 Distribution:

In their native habitat, both of these frogs inhabit South-east Australia, in particular Southern New South Wales, Victoria and Tasmania (Gartside, 1982; Lauck *et al*, 2005; Heard *et al*, 2008; Wassens, 2008). The natural distribution of *L. ewingii* has remained stable, despite influences from anthropogenic sources (Ficken & Byrne, 2012). However the natural distribution of *L. raniformis* has declined. Originally widespread throughout Southern New South Wales, it is now listed as endangered and populations have been reduced to sites near or beside the Murrumbidgee and Murray rivers (Pyke, 2002). It has also experienced distribution reductions in Victoria and Tasmania.



Despite the declines in Australia, both these frogs have been successfully introduced and naturalised into New Zealand. *L. ewingii* was introduced and released into New Zealand in Greymouth in 1875. The species then became widespread in the South Island, with a large population existing on the West Coast (Alderton, 1985). *L. raniformis* was introduced to Canterbury, New Zealand in 1867, then again into Southland in 1868. Several translocations took place within New Zealand and *L. raniformis* was introduced to Napier in 1888 (Voros *et al*, 2008). Due to these human mediated introductions both species of exotic frog have become well established and distributed. An electronic atlas of their current distributions published by the Department of Conservation (DoC) shows that *L. ewingii* is widespread in the South Island and Stewart Island and in the North Island, mainly occurring from Wellington to Palmerston North. Small pockets of populations also exist scattered around the North Island. *L. raniformis* is now widespread throughout the whole of New Zealand (<http://www.doc.govt.nz/our-work/reptiles-and-frogs-distribution/atlas/>). An examination of the distributions of these frogs in the South Island of New Zealand shows an interesting difference in their distribution, *L. raniformis* is found more in the coastal, low lying areas of the South Island while *L. ewingii* is found more predominantly in higher altitude alpine areas (Fig 1.1). This difference in their distribution in the South Island becomes an important basis for this thesis.



**Fig 1.1** Distribution maps of *L. raniformis* on the left and *L. ewingii* to the right. Adapted from <http://www.doc.govt.nz/conservation/native-animals/reptiles-and-frogs/reptiles-and-frogs-distribution-information/electronic-atlas/>

### 1.3 Population Declines:

Amphibian populations are declining worldwide (Houlahan *et al*, 2000). One known cause of amphibian population decline worldwide is chytridiomycosis.

Chytridiomycosis is a disease caused by what is known as amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Skerratt *et al*, 2007). *Litoria* frogs have been shown to be susceptible to chytridiomycosis (Ohmer *et al*, 2013), however as a result of lack of monitoring, due to being an exotic species, the effects of chytridiomycosis on New Zealand populations of *Litoria* frogs is relatively unknown (Shaw *et al*, 2014).

Work into the relationship between humans and amphibians has found that amphibian community assemblage is related to extinction and colonization.

Degradation of ecological features and alteration of landscapes can cause the extinction of many sensitive species (Ficetola & Bernardi, 2004). Ficetola & Bernardi (2004) also conclude that only mobile and resilient species can migrate between habitat patches and recolonise them. Increased land use by humans as a result of population growth results in the destruction and fragmentation of natural landscapes (Ficetola & Bernardi, 2004). Therefore in areas with high levels of human activities, natural habitats such as wetlands, lakes, forests and streams are being drained, altered and reduced to small and disconnected patches, these patches are then surrounded by areas that are used for agriculture and urban growth (Hazell *et al*, 2001; Hamer & Organ, 2008).

Human disturbance can have an effect on animals in terms of modifying behaviour and altering habitat use (Beale & Monaghan, 2004). Thus human disturbance can lead to behavioural costs such as reproductive failure and reductions in home range and territory size (Garner *et al*, 2008), as well as physiological costs, such as a reduction in immune response and survival (Choung *et al*, 2004). Work by Garner *et al* (2008) found that human disturbance as simple as foot traffic is enough to alter the behaviour and local abundance of recently hatched frogs. Their results showed that human disturbance altered the escape behaviour of these juvenile frogs, and that frog movement patterns were affected by human disturbance. They concluded that human disturbance is costly to juvenile frogs and that disturbance may cause fragmentation of local populations.

Heavy metal pollution in freshwater systems as a result of industrial and agricultural practices may have a large effect on species inhabiting freshwater habitats (Greig *et al*, 2010). Work by Ficken & Byrne (2012) found that species richness was negatively correlated with sediment concentrations of heavy metals and that distribution of the three most common species of frog in southeastern Australia, including *L. ewingii*, were significantly negatively associated with total levels of metal contamination at individual sites.

Pesticides have also been shown to have an effect on the development and growth of frogs. Choung *et al* (2011) found that highly modified areas, such as agricultural fields for crop production provide breeding habitat for *L. raniformis*. However, this exposes these frogs, during critical stages of their development, to pesticides used on rice and crops. This study found that tadpoles exposed to the major breakdown products of insecticides had significantly slowed larval development and delayed metamorphosis. This result indicates a risk posed by agricultural pesticides to the growth and development of the endangered *L. raniformis*.

Despite the degradation of natural habitats through conversion to urban and agricultural land, some frog species have adapted and are able to make use of man-made structures such as farm dams as suitable habitat (Hazell *et al*, 2001). A further study by Hazell *et al* (2004) compared use of constructed habitat and natural habitat by several frog species in Australia. The study found that farm dams supported similar numbers of frog species to natural ponds, although there were differences in the assemblages of species. The results also showed that water bodies with high levels of vegetation and lack of predators is likely to support a higher number of frog species regardless of whether it is man made or natural. However, they also stress the need to conserve natural environments for species that are unlikely to use man made areas for habitat.

#### **1.4 Conservation:**

*L. ewingii* is classified as 'Least Concern' by the IUCN, with its population trend considered stable. However, *L. raniformis* is considered endangered with a decreasing population trend. However, both species have become widespread and abundant in New Zealand (Newman *et al*, 2010).

Habitat development and enhancement have been suggested as vital and important components for the recovery strategies of frog species (Pyke, 2002). The level of disturbance is an important aspect of their habitat (Garner *et al*, 2008), as both of these frog species are known to inhabit areas near man made structures (Hamer & Organ, 2008). Pyke (2002) suggests the need for a better understanding of the factors that control distribution and abundance, which would lead to knowledge that may benefit the conservation of frog species.

Finally a paper by Bishop (2008) highlights the potential benefits that these New Zealand populations of frogs have for study. There are certain barriers that are in place which can restrict or prevent work being done on species that are considered endangered or protected, thus work often has to be done on similar or related species and then conclusions are drawn from those results. Bishop (2008) suggests that barriers such as that may be avoided by using the well-established populations of *Litoria* frog in New Zealand as study subjects.

### **1.5 Temperature Acclimation:**

Temperature is an important environmental factor that can have large physiological and behavioural effects on animals in the wild (Brattstrom, 1968). A change in temperature outside of an animal's optimum or preferred temperature can have an effect on their physiology. Thermal acclimatisation is a mechanism that allows an organism to survive adverse conditions that may occur in their habitat (Brattstrom, 1970). Early work on thermal acclimation in amphibians has shown that species with small geographic ranges have a poor ability to thermally acclimate, and that species that can compensate to thermal changes the most have the widest geographic ranges (Brattstrom, 1970). Work by Putnam & Bennett (1981) showed that changes in behavioural performance when temperature was altered were almost instant and that there was no acclimation of performance after 7 days of acclimation.

However, there is debate over whether thermal acclimation is actually present in amphibians. Feder *et al* (1984) suggest that early experiments involving thermal acclimation may have not accounted for confounding factors, which may have altered results. These variables may include feeding, fasting and insufficient duration of acclimation. They advise caution in the design of experiments involving the use of thermal acclimation.

It seems that the ability to thermally acclimate may come down to each individual species, or even life stages within a species. Tadpoles of the frog *Limnodynastes peronii* have been shown to be able to thermally acclimate their locomotor performance (Wilson & Franklin, 1999), however the adult of that same species does not have the ability to thermally acclimate locomotor performance (Wilson & Franklin, 2000). In a different species, *Xenopus laevis*, both the tadpoles and the adults show the ability to thermally acclimate their locomotor performance (Wilson *et al*, 2000).

### **1.6 Respiration:**

Temperature has been shown to have a large effect on the body. A lowering of body temperature will slow down the rate of biochemical reactions, while increasing body temperature will have the opposite effect (Heldmaier & Ruf, 1992). Metabolism is the process of turning fuel into usable energy (ATP). The changes in the rate of biochemical reactions as a result of temperature can be measured as a change in metabolism. The most common method of measuring a change in metabolism in ectothermic animals is through oxygen consumption (Feder, 1982). Another useful way to express the temperature dependence of a process, in this case, respiration, is through Q<sub>10</sub>. The Q<sub>10</sub> (temperature coefficient) is a measure of the rate of change of a biological system as a result of an increase in temperature by 10°C. However, the ability to thermally acclimate as determined using metabolic rate seems to vary in organisms from temperate and tropical regions. The general view is that amphibians from tropical areas have less thermal acclimation ability; while temperate amphibians can often show significant changes in metabolism once they have been thermally acclimated (Feder, 1982).

Early work on the effect of temperature on oxygen consumption in toads showed that temperature acclimation had a small effect on metabolic rates (Carey, 1979). However, it has now been shown that the ability of some amphibians to thermally acclimate may be dependent on the season. Work by Rocha & Branco (1998) showed that the rate of oxygen consumption in the bullfrog, *Rana catesbeiana*, during winter was lower than summer when measured at 10, 15, 25 and 35°C. Similar work done on the toad *Bufo paracnemis* showed that oxygen consumption was higher during summer when toads were kept at 25°C (Bicego-Nahas *et al*, 2001). Tropical

and subtropical amphibians are generally considered to lack the ability to thermally acclimate (Feder, 1982), however, work by Chang & Hou (2005) showed that metabolic rate of the subtropical frog, *Rana latouchii*, was affected by time of day, season, and acclimation temperature. They showed that summer males had higher oxygen consumption values than frogs of other seasons when measured at 15, 20, 25 and 30°C when adjusted for body mass and acclimation temperature. Their results support the hypothesis that thermal acclimation of metabolism is seasonally dependent, which has not been shown in other tropical amphibians.

Recent work done in Australia on the frog *Limnodynastes peronii* has shown that the tadpoles of this species do not thermally acclimate metabolic performance in terms of heart rate, oxygen consumption and locomotion when comparing tadpoles raised in stable (24°C), narrowly variable (22-26°C) and widely variable (14-34°C) thermal treatments. This work conducted by Niehaus *et al* (2011) found that oxygen consumption did not differ between tadpoles raised in stable or variable thermal conditions and that oxygen consumption was virtually unaffected when measured over a thermal range. They show that these tadpoles show little ability to alter their metabolic traits to thermal variability and that this has implications for the growth and population dynamics of frogs in their environment.

There are very few studies that have examined the effect of temperature on the physiology of the frogs *L. ewingii* and *L. raniformis*. Cree (1984) examined the effects of temperature on the oxygen consumption of these two species and found that at 23°C both frogs remained alert and had similar rates of oxygen consumption. However, at 7°C *L. raniformis* became torpid and oxygen consumption was significantly lower than that of *L. ewingii*. This difference in physiology as a response to temperature may have important implications in terms of the distribution of these species.

### **1.7 Locomotion:**

Body temperature has a large influence on the rate of all physiological processes. Animals, such as amphibians, may be subjected to changes in body temperature as a result of the time of day or season (Putnam & Bennett, 1981). Locomotor performance has been shown to be thermally dependent, and most amphibians do not

have the ability to acclimate their locomotory performance to cold temperatures (Bennett, 1990).

Early work on the effects of temperature on locomotion showed that changes in body temperature had an almost instant effect on locomotion, and that thermal acclimation to certain temperatures had no effect on locomotory performance (Putnam & Bennett, 1981).

There are many studies that demonstrate the ability of fish to acclimate their locomotory capabilities to different temperatures (Beddow & Johnston, 1995). However, by comparison, studies of temperature acclimation of locomotor performance in amphibians are lacking. A study conducted by Wilson & Franklin (1999) was the first to show acclimation of an amphibian's locomotor system to differing temperatures. They showed it in the tadpoles of the frog *Limnodynastes peronii*. They found that the maximum swimming speed was greater for the tadpoles acclimated to 10°C when tested against tadpoles acclimated to 24°C in 10°C and 24°C water. Further study by Wilson & Franklin (2000) was to see if this thermal acclimation of locomotor performance was present in the adult stage of *Limnodynastes peronii*. They acclimated the frogs for 8 weeks at two different temperatures. Jumping and swimming performance was then measured over a range of temperatures. They found no significant difference between the two groups that were tested. The aquatic frog *Xenopus laevis* was found to be able to thermally acclimate its locomotor performance in both its larval and adult life stages (Wilson *et al*, 2000).

Locomotion plays an important role in migration, mating, escaping predation and foraging for food (Bennett, 1990). Frogs often inhabit areas that are subjected to large variations in temperature, thus the inability to acclimate locomotion to temperature may therefore restrict movement patterns, as well as hinder escape from predators (Wilson & Franklin, 2000).

### **1.8 Aims of the thesis:**

In this thesis I will aim to provide a context about two exotic frogs from Australia, while also giving information on the background research on these frogs as well as other species of amphibian. The main objective of this study is to investigate the physiological responses of these two exotic frogs to acclimation at differing

temperatures and to determine if there are any significant differences between the two species, and whether this may control distribution. These frogs have become well established in New Zealand and this research will help provide information on their physiology in relation to temperature, an area that is lacking in information. This research may also provide information that may aid in the conservation of these frogs in their native Australia.

Hypothesis: Temperature is the main driver determining distribution of two Australian frogs.



## 2. General Methods

### 2.1 Study Species:

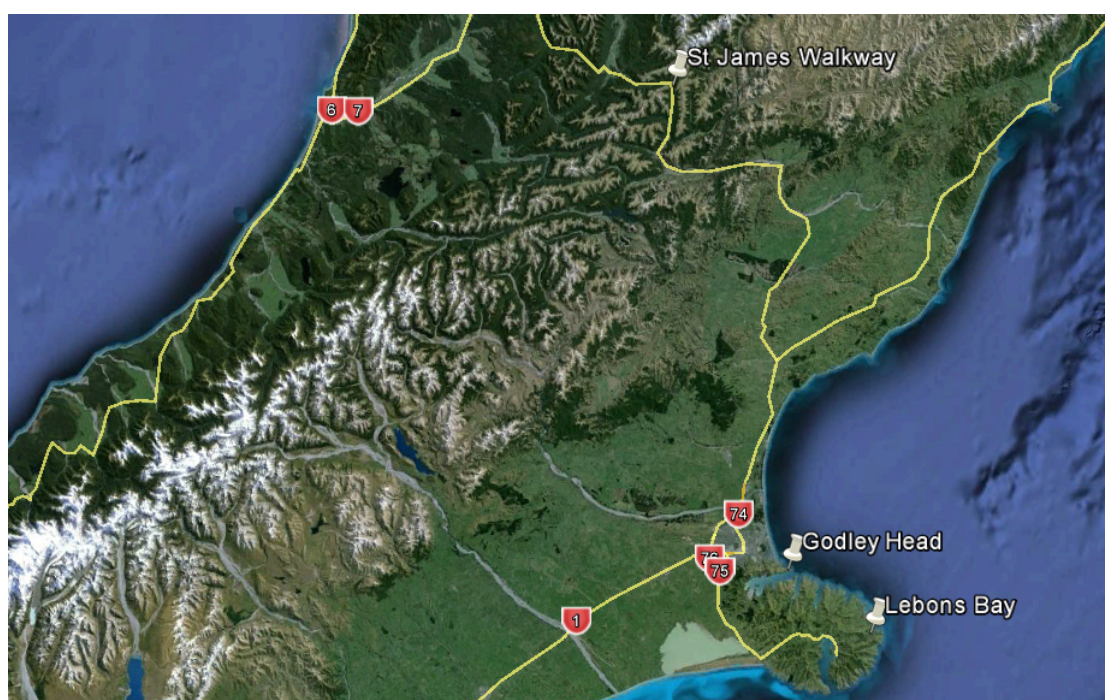
*Litoria raniformis* (Southern Bell Frog) (Fig 2.1) and *Litoria ewingii* (Brown Tree Frog) (Fig 2.2) were the two species of frog used in this experiment. They were collected from ponds in the Canterbury area of New Zealand (Fig 2.3). *L. raniformis* was found primarily in two locations on Banks Peninsula, Canterbury, New Zealand: LeBons Bay (-43.747778, 173.087639) and Godley Head (-43.589160, 172.799244). *L. ewingii* was found in the Lewis Pass area of Canterbury, New Zealand. Collection took place at night at a pond near the St James Walkway (-42.379943, 172.401556). Nine adult frogs were collected from each species and brought back to be maintained at the University of Canterbury. While at the University of Canterbury both frog species were kept in large rectangular bins that contained moss beds, rocks and a supply of water (Fig 2.4; 2.5). These bins were placed in the corner of the University's aquarium that also has an automatic day/night cycle. Each frog was weighed and their snout-to-vent length (SVL) was recorded. Sex of each individual frog was not recorded.



**Fig 2.1** *Litoria raniformis* (Southern Bell Frog). Photo credit: Emma Foster



**Fig 2.2** *Litoria ewingii* (Brown Tree Frog). Photo credit: Emma Foster



**Fig 2.3** Location of collection sites in the Canterbury region. Adapted from Google Earth. Photo credit: Scott Kelly



## 2.2 Acclimation:

Acclimation to certain temperatures can play an important role in whether an animal is capable of adjusting its physiology to best suit that environment. An animal that can acclimate will often survive better in a habitat with a changing temperature. During acclimation frogs were placed in large bins (Fig 2.4) that contained rocks, a moss bed and a supply of water. The supply of water was changed whenever water got too low or became too dirty; similarly the moss bed was replaced monthly. These large bins were then placed in temperature-controlled rooms (5, 15, 25°C) on a twelve-hour light/dark cycle for a month. These three temperatures were used for acclimation as they fall within the ‘normal’ temperature range that these frogs would experience in the wild, without going too far to either temperature extremes. The time frame of a month for acclimation was used due to time constraints of the thesis, as well as one month or longer being a conventional time in the literature (Wilson & Franklin, 2000; Kolbe et al, 2010; McCann et al, 2014) During this time frogs were fed on a diet of mealworms and fruit flies. Feeding was stopped one week before the end of the acclimation period in order to get as close to basal metabolic rate as possible.



**Fig 2.4** Bins that the frogs were housed in at University of Canterbury. Photo credit: Emma Foster



**Fig 2.5** Photo of the inside of the bin housing *L. raniformis* with rocks, water and moss bed. Photo credit: Emma Foster

### 2.3 Respiration:

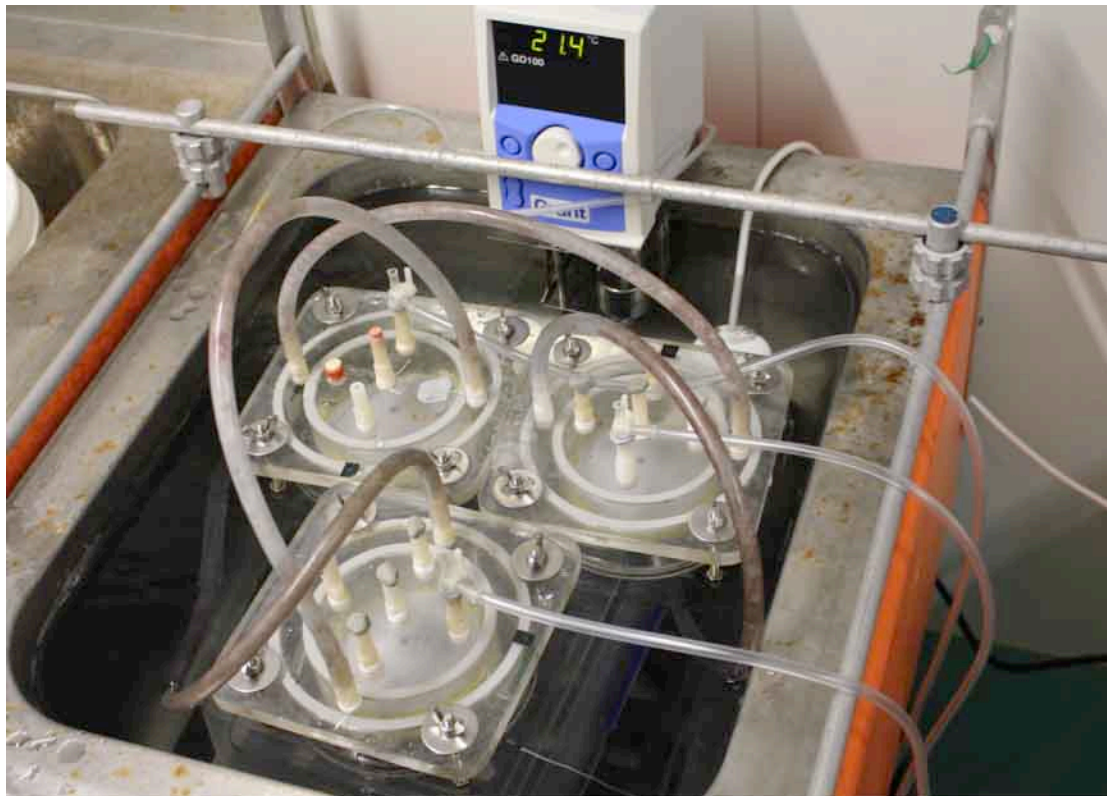
Respiration was used to determine metabolic rate and whether acclimation had any effect on the physiology of the two study species of frogs. After a month of acclimation at a certain temperature 3 frogs at a time were brought into the lab in order to conduct experiments. The frogs were left in air filled respiration chambers (Fig 2.7) in a water bath overnight that had a flow of air being pumped through them in order to let the frogs settle, the water bath temperature was set to the same temperature as their acclimation temperature (Fig 2.6). A water bath was used in order to keep the temperature of the air inside the respiration chamber at a constant and stable level, while also allowing for acute temperature changes during experimentation. Control measurements were taken on empty chambers that showed that there was no change to oxygen and carbon dioxide levels. This indicated that there was no inherent drift in the set-up and that there was no microbial activity in the respirometers. At the beginning of each experiment an ADInstruments ML250 Gas analyser connected to Powerlab using Labchart version 7.2.5 was zeroed using nitrogen and then calibrated using a supply of 5% carbon dioxide in air. To start the experiment a 10ml sample of air was taken from the chamber and put into the gas

analyser in order to determine oxygen and carbon dioxide levels. The chamber was then sealed and samples were taken every half hour for one and a half hours. After sampling was completed at the acclimation temperature the chambers were flushed with air and the temperature of the water bath was altered randomly to one of the test temperatures. The frogs were then left for an hour so that the chamber temperature matched the water bath temperature. Once the temperatures had equilibrated then the sampling process was repeated. The acute temperature changes used for this experiment were 5, 10, 15, 20 and 25°C. This process was repeated for all frogs of both species after each month long acclimation period. For experimentation with *L. ewingii*, the volume of the respiration chambers were halved using plastic blocks to get a more accurate reading for the smaller frogs.



**Fig 2.6** Experimental setup for respiration experiments showing the water bath, respiration chambers and air supply. Photo credit: Emma Foster





**Fig 2.7** Respiration chambers used during both respiration and locomotion experiments showing the temperature control and air supply. Photo credit: Emma Foster

## 2.4 Locomotion:

Locomotion plays an important role in an amphibian life cycle and has also been shown to be thermally dependent. Thus assessment of these two frogs' locomotor abilities was used as an indicator to determine whether thermal acclimation took place. After a month of acclimation to a set temperature three frogs were brought to the lab in order to perform experiments on their jumping capabilities. Frogs were placed inside a respiration chamber and left for one hour in order to settle with a supply of air being pumped through the chamber (Fig 2.7). The respiration chamber was set in a water bath with a temperature matching the acclimation temperature. After an hour a frog was removed and placed in the center of a 1.6m x 1.6m jumping platform (Fig 2.8). This frog was made to jump at least eight times in order to determine a maximal jump distance. Markers were placed at the location of each jump and distance was recorded. Gentle pinching of the urostyle with forceps was used to

elicit jumps from the frogs when necessary. Once the first set of jumps were completed the frogs were placed back inside the respiration chambers and the temperature of the water bath was adjusted, the frogs were then left for an hour for the temperatures to equilibrate and the process was repeated. The temperatures used for this experiment were 5, 10, 15, 20 and 25°C. This was done for all frogs of both species.

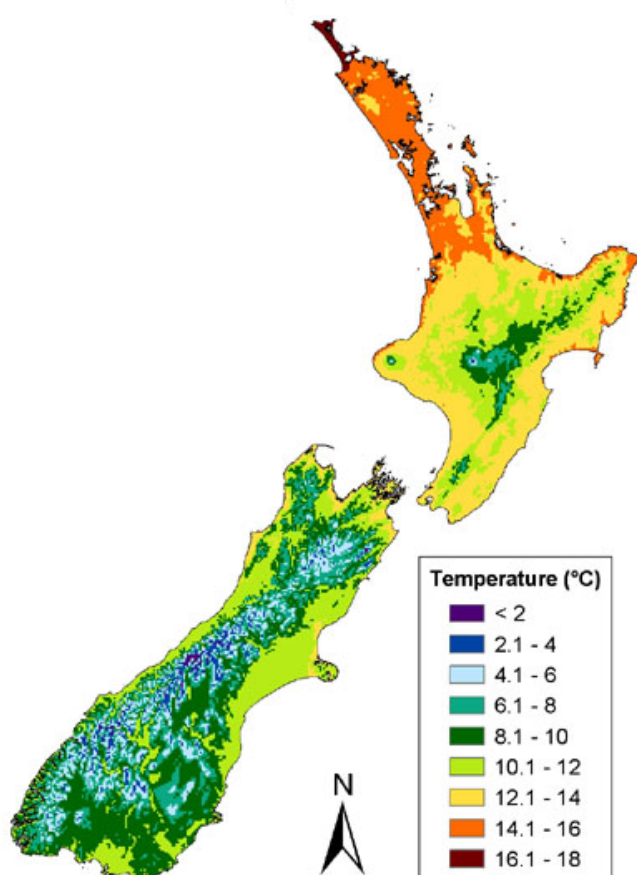


**Fig 2.8** Jumping platform used for locomotion experiments (1.6m x 1.6m). Circles indicate intervals of a distance of 10cm from the center of the platform. Photo credit: Emma Foster

### 3. Respiration

#### 3.1 Introduction:

*L. raniformis* and *L. ewingii* were introduced to New Zealand over 100 years ago (Gill, 1973; Cree 1984). Since their introduction they have become well established and abundant. Despite *L. raniformis* experiencing population declines in its native Australia, very little research to do with their physiology has been conducted on these frogs, either in Australia or here in New Zealand. Most research that has been conducted in Australia has focused on population declines (Pyke, 2002; Wassens, 2008) and anthropogenic influences on habitat and population structure (Hamer & Organ, 2008), while in New Zealand; research has mostly focused on distribution (Alderton, 1985). Very little work has been centered on the biology and physiology of these two species, despite the call for more basic biological research (Bishop, 2008). Physiological research that has been conducted on these two frogs in New Zealand



**Fig 3.1** Mean annual temperature (°C) in New Zealand, 1971-2000. Adapted from:  
[https://www.niwa.co.nz/sites/niwa.co.nz/files/sites/default/files/images/climate\\_-\\_nz\\_temperature\\_1971-2000.jpg](https://www.niwa.co.nz/sites/niwa.co.nz/files/sites/default/files/images/climate_-_nz_temperature_1971-2000.jpg)



has focused on the biology and physiology during larval development (Cree, 1984), as well as showing the ability of *L. ewingii* to tolerate freezing (Bazin *et al*, 2007).

Individuals of both species of frog are exposed to a wide range of environmental conditions during their life span. They may be found in water, on the ground near a water source or on low vegetation surrounding a water source (Pyke, 2002). It has also been observed that non-breeding *L. ewingii* males can be found considerable distances from a water source (Cree, 1984). *L. raniformis* may be active during the day or night, while *L. ewingii* is predominately active during dusk/night. During these times they can be exposed to a variety of temperatures ranging from 0°C to 30°C. In the South Island of New Zealand these frogs occupy differing habitats, with *L. raniformis* occurring more in the temperate/coastal areas and *L. ewingii* occurring in more alpine regions (Fig 1.1). Despite the difference in distribution patterns individuals of each species have been found across both habitats. A key difference between these two habitats is the average temperature during the year; data from the National Institute of Water and Atmospheric Research (NIWA) shows that on average *L. raniformis* is exposed to a mean annual temperature of 10.1-14°C, while *L. ewingii* is exposed to a mean annual temperature of 4.1-10°C (Fig 3.1). Therefore it becomes important to investigate what effect temperature has on these two related species and whether a difference in temperature physiology is accounting for this difference in distribution.

These populations of frogs in New Zealand provide a unique opportunity for study, as the restrictions that are in place to protect them in Australia do not apply in New Zealand (Bishop, 2008). Thus research on the physiology of these frogs in New Zealand can aid in the conservation of these frogs in their native Australia. My research will aim to expand on previous work as well as fill some gaps in the knowledge of the physiology of these two frogs while also providing some context as to why we see differences in their distributions across New Zealand.

### 3.2 Methods:

For methods used during the respiration portion of this thesis refer to General Methods 2.1, 2.2 and 2.3.

After experimentation statistical analysis was carried out on the data using Excel and GraphPad Prism version 6. All oxygen consumption measurements were

adjusted for weight of the frog as well as volume of chamber occupied by the frog. One-Way ANOVA was used in order to determine whether acclimation to a certain temperature had an effect on oxygen consumption at differing acute temperatures, while a Two-Way Repeated Measures ANOVA was done to compare the intra- and interspecific oxygen consumption of both species at certain temperatures after acclimation was completed.

### **3.3 Results:**

#### **Observations during acclimation: *L. raniformis***

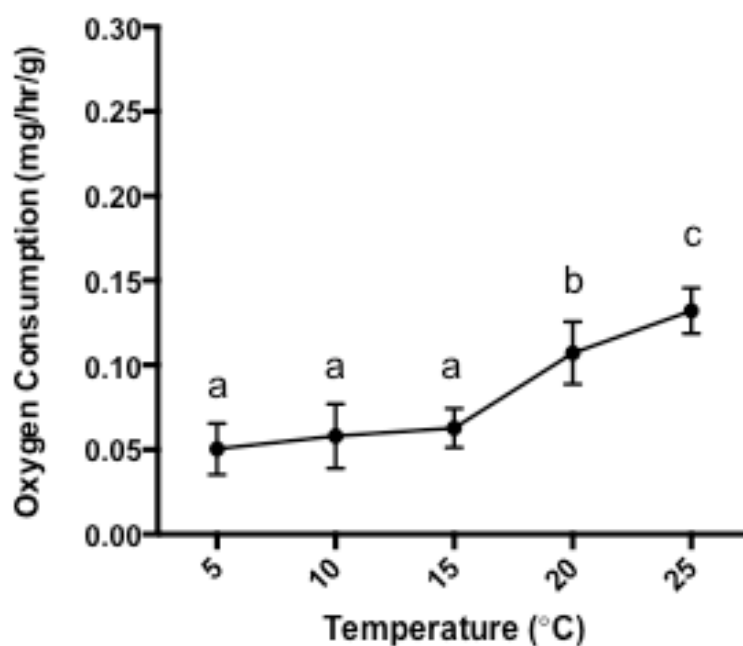
*L. raniformis* was first acclimated to 15°C; during this time the frogs were alert and responsive. They split their time evenly between the rocks, water and moss provided. During feeding almost all of the mealworms and flies provided were eaten with very little being left the following day, water was replaced weekly or when it became too dirty. During acclimation to 25°C the frogs spent most of their time in either the water or the moss bed. Frogs were alert and active during this time, however, there was one fatality due to a frog escaping from the enclosure and dehydrating. Water was replaced every two days due to evaporation. Frogs ate all food provided for them and seemed to cope well at 25°C. At 5°C the frogs were lethargic and unresponsive. They spent most of their time in or on the moss bed and would barely move when touched. Water was changed weekly despite the frogs spending minimal time in the water. Food that was provided was only eaten in small quantities. While the frogs seemed lethargic at 5°C they appeared not to be in any distress due to the low temperature.

#### **Observations during acclimation: *L. ewingii***

*L. ewingii* was initially acclimated to 15°C; frogs were alert during this time and responsive to touch and sound. Frogs were often found beneath the moss bed but also spent time on the walls of the enclosure and on the edge of the water provided. Frogs were fed mostly on fruit flies but were given some smaller mealworms every couple of weeks, all food that was provided was eaten. During acclimation to 25°C frogs became highly alert, responding immediately to almost every stimulus. This excitability caused one frog to try burrow under a rock where it got stuck and died overnight. Water in the enclosure was changed every three to four days. Frogs were

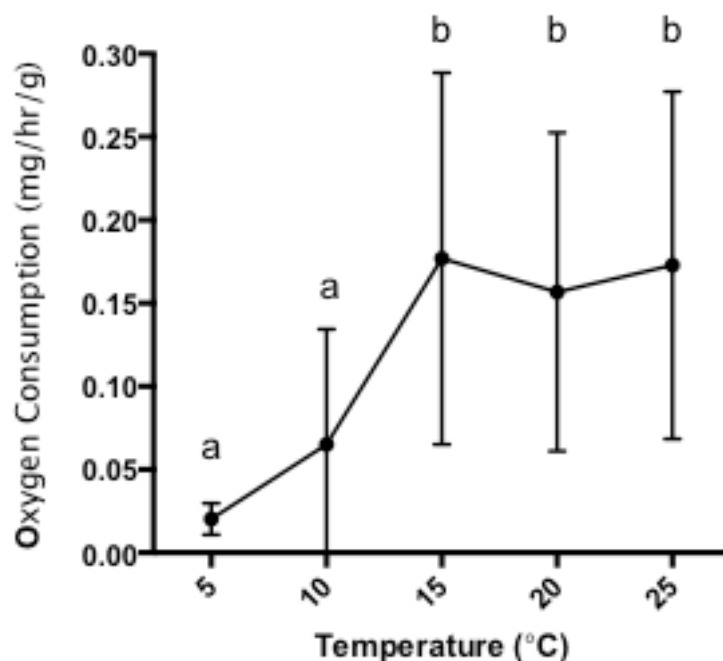
fed fruit flies and despite their excitability seemed relatively comfortable at 25°C, frogs ate all food that was provided. At acclimation to 5°C frogs remained alert and responsive, however, they spent the majority of their time under the moss. Two frogs were found deceased during the acclimation process, they were found dehydrated on top of the moss bed provided. Frogs were fed fruit flies during the acclimation process, though very little food was eaten during acclimation. Despite the cold temperature the frogs appeared to be capable of functioning at 5°C.

### Respiration: *L. raniformis*



**Fig 3.2** Effect of temperature on oxygen consumption of *L. raniformis* after acclimation to 5°C. n=8. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ).

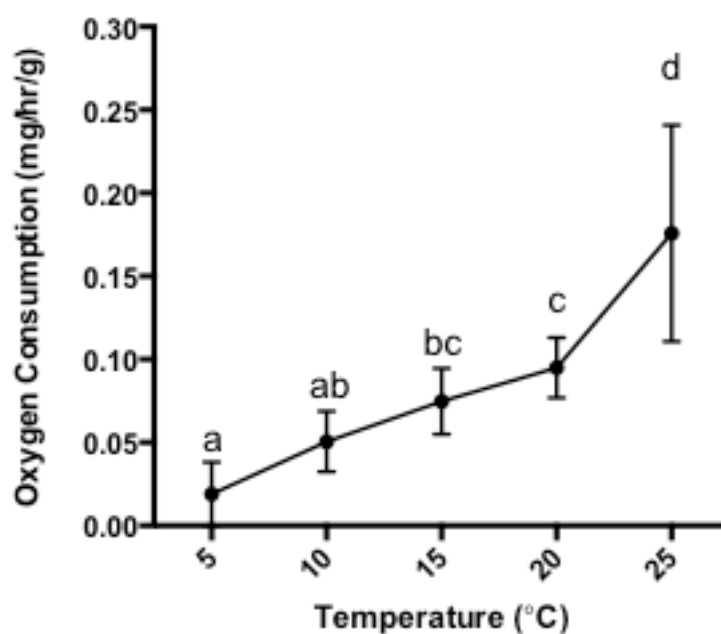
Analysis of oxygen consumption of *L. raniformis* acclimated to 5°C showed that there was a significant difference in oxygen consumption with relation to change in temperature ( $P < 0.001$ ). A Tukey's multiple comparisons test showed that oxygen consumption at 20°C and 25°C differed significantly from oxygen consumption at all other temperatures. Oxygen consumption at 5, 10 and 15°C did not differ significantly from each other (Fig 3.2).



**Fig 3.3** Effect of temperature on oxygen consumption of *L. raniformis* after acclimation to 15°C. n=9.

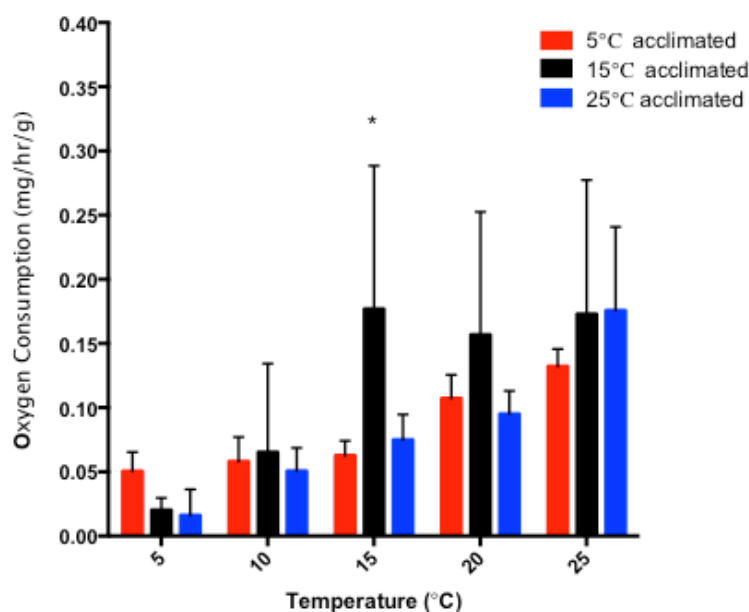
Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). 1 error bar clipped at axis limit.

An ANOVA of the oxygen consumption of the frog *L. raniformis* after acclimation to 15°C showed that there was a significant difference in oxygen consumption when exposed to differing temperatures ( $P < 0.001$ ). A post-hoc Tukey's test was conducted in order to determine at which temperatures oxygen consumptions differed from the others. Oxygen consumption at 15, 20 and 25°C differed significantly from 5 and 10°C (Fig 3.3).



**Fig 3.4** Effect of temperature on oxygen consumption of *L. raniformis* after acclimation to 25°C. n=8. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). 1 error bar clipped at axis limit.

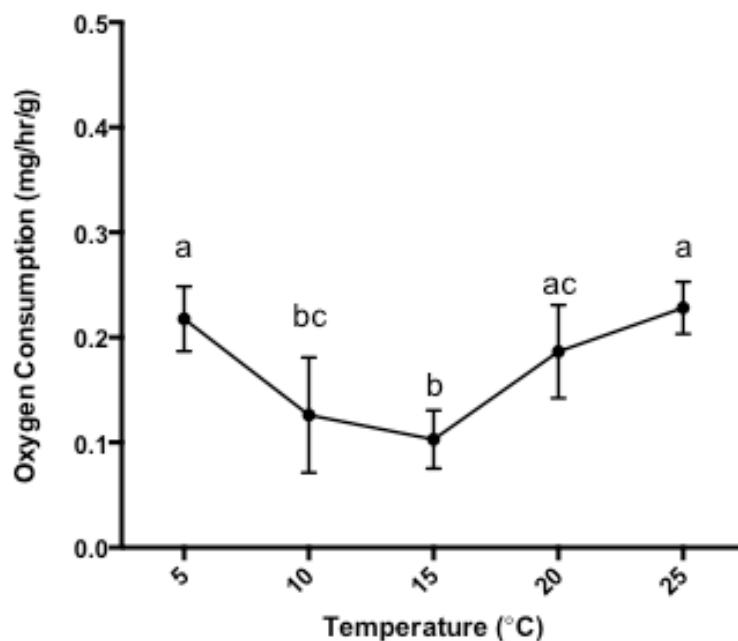
There was a significant difference in oxygen consumption of *L. raniformis* after acclimation to 25°C when exposed to differing temperatures ( $P < 0.001$ ). A multiple comparisons test showed that oxygen consumption at 25°C was significantly different than oxygen consumption at all other temperatures. Oxygen consumption at 5°C was significantly different from oxygen consumption at 15°C and 20°C, while oxygen consumption at 10°C was significantly different from 20°C (Fig 3.4).



**Fig 3.5** Comparison of the oxygen consumption of *L. raniformis* after acclimation to three differing temperatures (5, 15, 25°C). Error bars represent standard error. (\*) represent significant difference of oxygen consumption ( $\alpha = 0.05$ ).

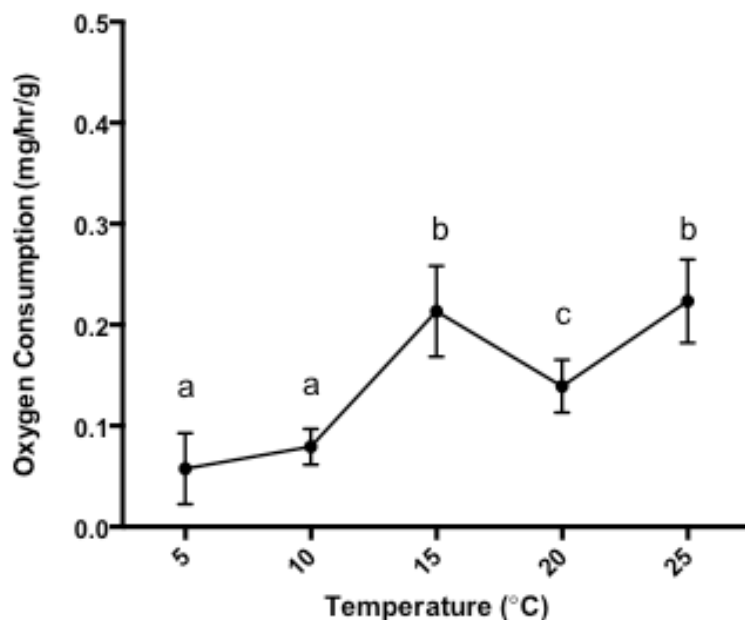
A Two-Way ANOVA was conducted in order to determine the levels of difference in oxygen consumption of *L. raniformis* after acclimation to three differing temperatures of 5°C, 15°C and 25°C. The results of the Two-Way ANOVA show that there was a significant difference in the levels of oxygen consumption after acclimation for a month to three different temperatures. A multiple comparisons test was carried out in order to determine which levels of oxygen consumption differed at differing acute temperatures. The test revealed that oxygen consumption for *L. raniformis* acclimated to 15°C for a month was significantly higher at 15°C than when *L. raniformis* was acclimated to 5°C and 25°C. Oxygen consumption at 5°C, 10°C, 20°C and 25°C did not differ significantly with regard to differing acclimation temperatures (Fig 3.5).

### Respiration: *L. ewingii*



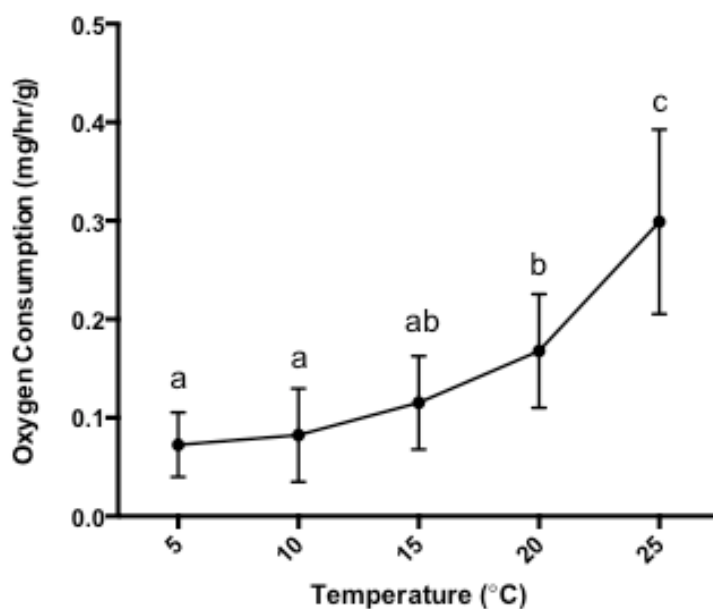
**Fig 3.6** Effect of temperature on the oxygen consumption of *L. ewingii* after acclimation to 5°C. n=6. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ).

A one-way ANOVA of the oxygen consumption of the frog *L. ewingii* after acclimation to 5°C showed that there was a significant difference in the oxygen consumption when *L. ewingii* were exposed to differing temperatures ( $P < 0.0001$ ). A post-analysis Tukey's test was run in order to determine which levels of oxygen consumption differed significantly from the others. The Tukey's test showed that oxygen consumption at 5°C, 20°C and 25°C did not differ significantly from each other. Oxygen consumption at 15°C differed significantly from all other temperatures bar 10°C, while 20°C was not significantly different from 10°C (Fig 3.6).



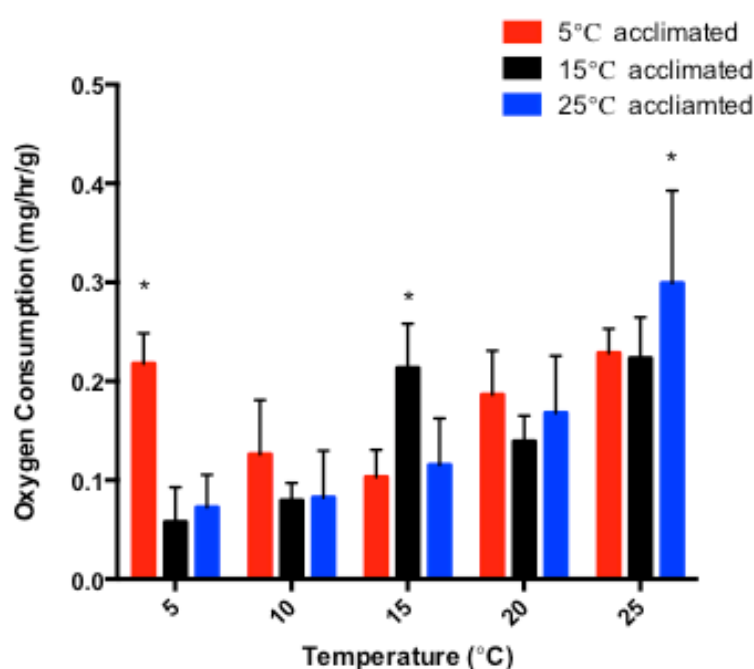
**Fig 3.7** Effect of temperature on the oxygen consumption of *L. ewingii* after acclimation to 15°C. n=9. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ).

Oxygen consumption of *L. ewingii* acclimated to 15°C was found to be significantly different in relation to change in temperature ( $P < 0.0001$ ). A Tukey's multiple comparisons test was conducted in order to determine which levels of oxygen consumption in relation to temperature differed. Oxygen consumption at 5°C and 10°C did not differ significantly from each other, while oxygen consumption at 20°C differed significantly from every other temperature. Oxygen consumption at 15°C and 25°C did not differ significantly from each other but were significantly different from all other temperatures (Fig 3.7).



**Fig 3.8** Effect of temperature on the oxygen consumption of *L. ewingii* after acclimation to 25°C. n=8. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ).

Analysis of the oxygen consumption of *L. ewingii* acclimated to 25°C revealed that there was a significant difference in the oxygen consumption in relation to change in temperature ( $P < 0.0001$ ). A Tukey's multiple comparisons test was used in order to determine which levels of oxygen consumption differed from each other. Oxygen consumption at 5°C, 10°C and 15°C did not differ significantly. At 20°C oxygen consumption differed significantly from 5°C and 10°C as well as 25°C, but not 15°C. Oxygen consumption at 25°C differed significantly from oxygen consumption at all other temperatures (Fig 3.8).



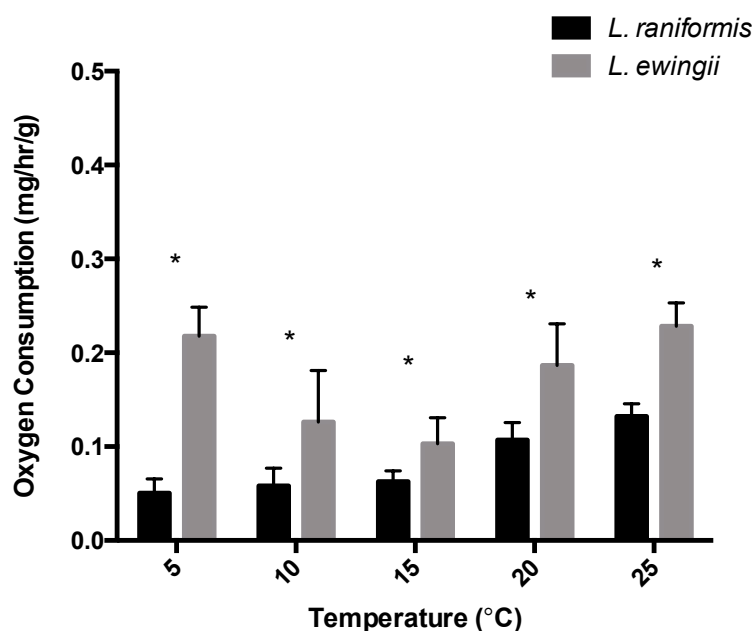
**Fig 3.9** Comparison of the oxygen consumption of *L. ewingii* after acclimation to three differing temperatures (5, 15, 25°C). Error bars represent standard error. (\*) represent significant difference of oxygen consumption ( $\alpha = 0.05$ ).

A Two-Way Repeated Measures ANOVA was carried out in order to determine how oxygen consumption of *L. ewingii* differed at a range of temperatures after frogs were acclimated for a month at three differing temperatures of 5°C, 15°C and 25°C. The results of the Repeated Measures ANOVA showed that there was a significant difference in oxygen consumption. The analysis revealed that acute temperature and the interaction of acute temperature and acclimated temperature were significant sources of variation ( $P < 0.0001$ ). A Tukey's multiple comparisons test was run in order to determine how oxygen consumption of *L. ewingii* differed after acclimation to set temperatures. At 5°C oxygen consumption of *L. ewingii* was significantly higher when acclimated to 5°C than when acclimated to 15°C or 25°C for a month.



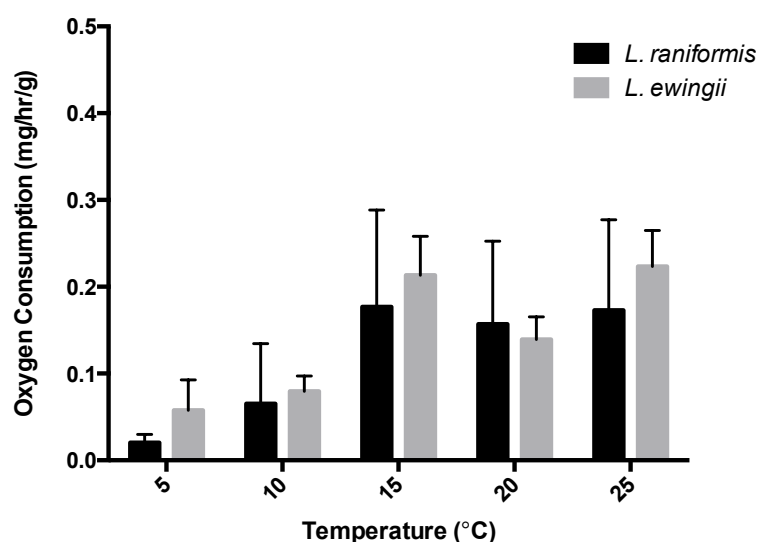
Oxygen consumption at 15°C was significantly higher when frogs were acclimated to 15°C for a month compared to when frogs were acclimated to 5°C and 25°C. At 25°C oxygen consumption of *L. ewingii* was significantly higher for frogs acclimated to 25°C for a month compared to frogs acclimated to 5°C and 15°C. Oxygen consumption at 10°C and 20°C did not differ significantly with regards to change in acclimation temperature (Fig 3.9).

### Respiration: Comparison of *L. raniformis* & *L. ewingii*



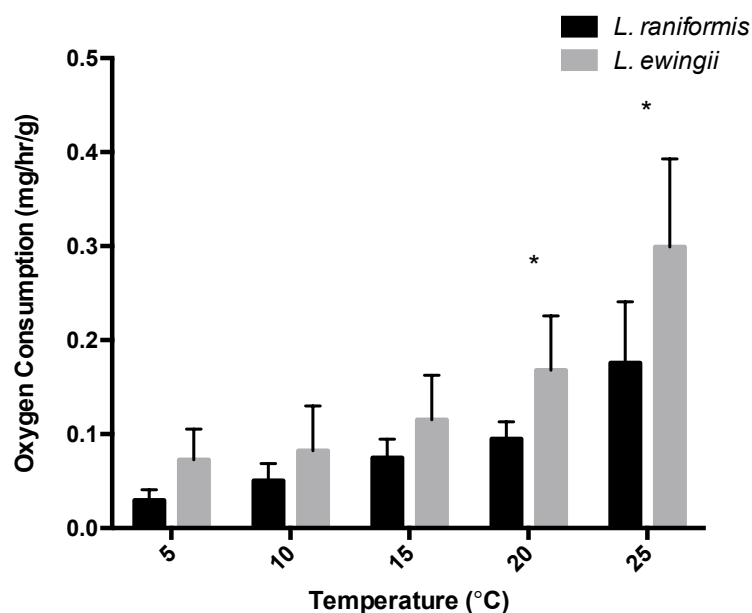
**Fig 3.10** Comparison of oxygen consumption of *L. raniformis* and *L. ewingii* acclimated to 5°C for a month. Error bars represent standard error. (\*) represent significant difference of oxygen consumption ( $\alpha = 0.05$ ).

A Two-Way ANOVA was done to determine at which acute temperature oxygen consumption of *L. raniformis* and *L. ewingii* differed with relation to acclimation temperature. The results showed that there was a significant difference in oxygen consumption between the two species after acclimation to 5°C for a month. Acute temperature ( $P < 0.0001$ ) and the interaction between acute and acclimated temperature ( $P = 0.0017$ ) were found to be significant sources of variation. A multiple comparisons test was conducted in order to see at which acute temperature oxygen consumption differed between the two species after acclimation to 5°C for a month. The test showed that there was a significant difference in oxygen consumption at all acute temperatures after acclimation. The results showed that oxygen consumption for *L. ewingii* was significantly higher than that of *L. raniformis* after acclimation (Fig 3.10).



**Fig 3.11** Comparison of oxygen consumption of *L. raniformis* and *L. ewingii* acclimated to 15°C for a month. Error bars represent standard error. (\*) represent significant difference of oxygen consumption ( $\alpha = 0.05$ ).

A Two-Way Repeated Measures ANOVA was carried out in order to determine the difference in oxygen consumption between *L. raniformis* and *L. ewingii* after acclimation for a month at 15°C. The ANOVA showed that there was a significant difference in oxygen consumption with acute temperature being a significant source of variation ( $P < 0.0001$ ). A multiple comparisons test was conducted in order to determine if there was any difference in oxygen consumption at acute temperatures between these two species. The test showed that there was no significant difference in oxygen consumption at any temperature after acclimation (Fig 3.11).



**Fig 3.12** Comparison of oxygen consumption of *L. raniformis* and *L. ewingii* acclimated to 25°C for a month. Error bars represent standard error. (\*) represent significant difference of oxygen consumption ( $\alpha = 0.05$ ).

A Repeated Measures ANOVA was carried out in order to determine the levels of oxygen consumption for *L. raniformis* and *L. ewingii* after acclimation to 25°C for a month. The results of the analysis showed that there was a significant difference in oxygen consumption and that acute temperature ( $P < 0.0001$ ) acclimated temperature ( $P = 0.0066$ ) and the interaction of acute and acclimated temperature ( $P = 0.0043$ ) were all significant sources of variation. A post-hoc multiple comparisons test was conducted to determine at which acute temperature there were significant differences in oxygen consumption with relation to acclimation temperature between the two species. The test showed that oxygen consumption was significantly higher for *L. ewingii* than for *L. raniformis* at 20°C and 25°C after acclimation to 25°C for a month. There was no significant difference in oxygen consumption between the two species at 5°C, 10°C or 15°C (Fig 3.12).

### 3.4 Discussion:

As an ectotherm, a frog's ability to feed, digest food, move, perceive predators, mate and predate all depends on access to temperatures that are suitable to that animal (McCann *et al*, 2014). Work has been carried out that shows that the geographic distributions of many species are correlated with temperature and thus may be constrained by the thermal regimes that are experienced in that habitat (Wilson, 2001; Sorte *et al*, 2011). This effect may be even more pronounced for amphibians as they are mostly active at night, and their increased vulnerability to desiccation from prolonged exposure to the sun prevents them from basking. Thus, thermoregulation in amphibians usually occurs by selecting suitable microhabitats (Navas *et al*, 2008).

Temperature has been shown to have a large effect on the physiological function of amphibians, as well as many other animals (Castellani *et al*, 2005), thus it becomes important to be able to measure and examine that effect in order to draw conclusions and implications about the effect temperature has. Respiration experiments are often used to examine the effect that temperature has on the metabolic function of an amphibian. Measuring oxygen consumption is a common way of examining a change in metabolism as a result of changes in temperature (Feder, 1982).

Early research on the effect of thermal acclimation on oxygen consumption concluded that amphibians from tropical areas have less thermal acclimation ability;

while temperate amphibians can often show significant changes in metabolism once they have been thermally acclimated (Feder, 1982). Early work on the effect of temperature on oxygen consumption in toads showed that temperature acclimation had a small effect on metabolic rates (Carey, 1979). However, research by Rocha & Branco (1998) revealed that the rate of oxygen consumption in the bullfrog, *Rana catesbeiana*, during winter was lower than summer when measured at 10, 15, 25 and 35°C. Similar work done on the toad *Bufo paracnemis* showed that oxygen consumption was higher during summer when toads were kept at 25°C (Bicego-Nahas *et al*, 2001). Tropical and subtropical amphibians are generally considered to lack the ability to thermally acclimate (Feder, 1982), however, work by Chang & Hou (2005) showed that metabolic rate of the subtropical frog, *Rana latouchii*, was affected by time of day, season, and acclimation temperature. They showed that summer males had higher oxygen consumption values than frogs of other seasons when measured at 15, 20, 25 and 30°C when adjusted for body mass and acclimation temperature. Despite the increase in academic attention to amphibians over the years, significant problems arise from the fact that most of the research has focused on species from the genus *Rana* and *Bufo*, thus the ability to make implications and draw conclusions about other species from these data is limited (Navas *et al*, 2008). Furthermore, this increase in research has not translated over to these two frogs *L. raniformis* and *L. ewingii*. A review of the biology of *L. raniformis* by Pyke (2002) cited very few papers when discussing the physiology of this frog. Similarly, research from New Zealand on the physiology of these frogs is very limited aside from work done by Cree (1984) and Bazin *et al* (2007). This work conducted by Cree (1984) also examined respiration physiology of tadpoles rather than adults, thus physiological information regarding the effects of thermal acclimation on the respiration physiology of the adult stages of both *L. raniformis* and *L. ewingii* is lacking. There is the need for more baseline physiological data on amphibians before more in depth research can be conducted. This is especially the case for conservation of an endangered species, thus it is hoped that this thesis will help provide more physiological data on these two frogs, as well as aid in the effort of their conservation.

The ability to acclimatise to a change in temperature in the environment allows certain animals the ability to live and thrive in places where other species may not be able to occupy, thus reducing competition for resources such as space and

food. This can become particularly important for species that are similar and therefore would share the same requirements in a given ecological niche. The goal of this experiment was to determine if the two Australian frogs *L. raniformis* and *L. ewingii* have the ability to thermally acclimate and whether this ability to acclimate may be contributing to differences seen in their distribution.

From the results for *L. raniformis* we can see that after acclimation to 15°C oxygen consumption is significantly higher at 15°C than 5°C or 10°C, however there is no significant difference in oxygen consumption at 15°C, 20°C or 25°C (Fig 3.3). This result shows that the frogs appear to be much more comfortable at 15°C, and thus are more active at that temperature than what would be expected from a standard Q10 curve. After acclimation to 25°C oxygen consumption was significantly higher at 25°C compared to all other temperatures (Fig 3.4). This graph shows that while there is a steady increase in oxygen consumption as temperatures increase, the significantly larger increase between 20°C and 25°C indicates that *L. raniformis* is much more comfortable functioning at the temperature that it has been acclimated to, than when it is exposed to other temperatures. Acclimation to 5°C shows a different story, in that there is no significant difference in oxygen consumption at 5°C, 10°C and 15°C. However, oxygen consumption does begin to increase significantly at 20°C and 25°C (Fig 3.2). This result appears to show a typical Q10 type curve, however the increase at 25°C is not as large as would be expected from the rest of the curve. It is likely that this result coincides with what can be seen in the literature in that at temperatures below 7°C *L. raniformis* enters a torpid state and thus was not able to fully acclimate to the low temperature (Cree, 1984). When comparing the oxygen consumption of *L. raniformis* after acclimation to all three temperatures of 5°C, 15°C and 25°C we can see that the only significant difference seen in oxygen consumption is at 15°C for the frogs acclimated to 15°C for a month (Fig 3.5). This result indicates that the only significant acclimation for *L. raniformis* occurred at 15°C, however the oxygen consumption for the frogs acclimated to 5°C, though not significantly, was higher at 5°C, than the frogs acclimated to 15°C and 25°C. Oxygen consumption is also higher at 25°C for the frogs acclimated to 15°C and 25°C compared to frogs acclimated to 5°C, though not significantly, however this may indicate that frogs are more active at this temperature that is more suited to them after acclimation.

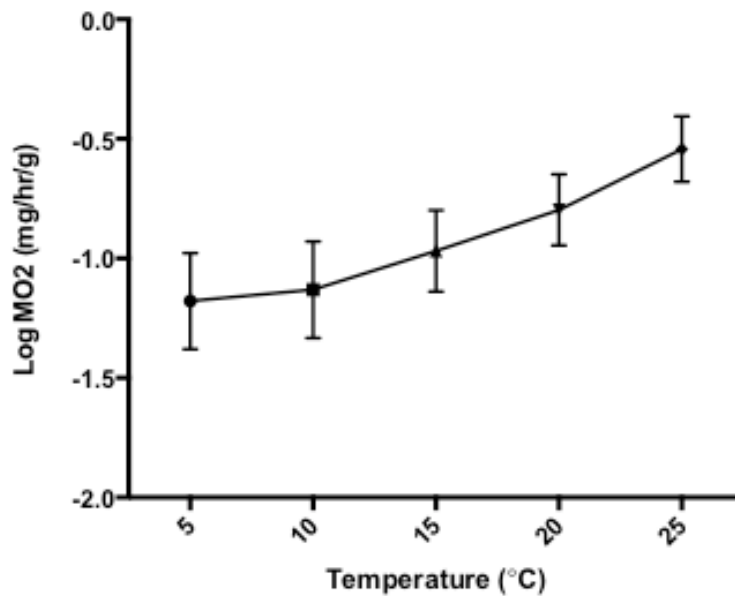
After acclimation to 15°C, oxygen consumption of *L. ewingii* was significantly higher at 15°C and 25°C, however oxygen consumption was

significantly lower at 20°C (Fig 3.7). This result shows that oxygen consumption for *L. ewingii* is higher than predicted from the rest of the curve indicating that the frogs are more active at their acclimation temperature. After acclimation to 25°C *L. ewingii* shows a much more standard temperature-dependent curve (Fig 3.8). A Log transformation of the oxygen consumption data shows a straight line, indicating that after acclimation to 25°C, *L. ewingii* show a standard Q10 response to an increase in temperature (Fig 3.13). After acclimation to 5°C, oxygen consumption starts off high at 5°C before dropping significantly at 15°C, before rising significantly at 25°C (Fig 3.6). This result indicates that at 5°C and 10°C *L. ewingii* is much more active, thus we are measuring active oxygen consumption rates, while at 15°C, 20°C, and 25°C they are much less active thus we are measuring true resting rates. The slight dip in oxygen consumption at 25°C mirrors that seen in *L. raniformis*. A comparison of oxygen consumption of *L. ewingii* after acclimation to 5°C, 15°C and 25°C shows that oxygen consumption is significantly higher at the temperatures at which the frog has been acclimated (Fig 3.9). This result indicates that these frogs have the ability to acclimate, which is shown by their significantly increased oxygen consumption at acclimated temperatures. Of particular importance is the fact that at 25°C the oxygen consumption of *L. ewingii* acclimated to 25°C is significantly higher than frogs acclimated to 15°C and 5°C, which is not the case for *L. raniformis*.

A comparison of oxygen consumption for both species of frog showed that there are no significant differences after acclimation for one month to 15°C (Fig 3.11), this result is not entirely unexpected as this temperature falls within the range that both species of frogs are likely to experience in their habitats. Comparing both species after acclimation for a month at 25°C shows that *L. ewingii* has significantly higher levels of oxygen consumption than *L. raniformis* at 5°C, 20°C and 25°C (Fig 3.12). This shows that after acclimation to 25°C *L. ewingii* is far more active at the higher temperatures, which is an unexpected result as *L. raniformis* is found more commonly in warmer areas (Fig 1.1) and thus would be expected to be more active at the higher temperatures. After acclimation of both species of frogs to 5°C for a month we can see that oxygen consumption is significantly higher for *L. ewingii* than *L. raniformis* at 5°C (Fig 3.10). This result coincides with what is indicated in the literature in that *L. ewingii* is a much more cold tolerant species, exemplified by its living in a cooler area of New Zealand (Fig 1.1; Fig 3.1) as well as being able to

survive freezing (Bazin *et al*, 2007), while *L. raniformis* has been shown to be unable to tolerate low temperatures (Cree, 1984).

From these data we can begin to draw some conclusions in that it appears that *L. ewingii* possesses the ability to thermally acclimate and shift its metabolic rate to allow it to function optimally at a given temperature, while *L. raniformis* seems to only be able to acclimate its metabolic rate to higher temperatures.



**Fig 3.13** Log conversion of oxygen consumption for *L. ewingii* acclimated to 25°C for 1 month. Error bars show standard error.

## 4. Locomotion

### 4.1 Introduction:

Body temperature is known to have a large effect on the rate of physiological processes. Ectotherms, such as amphibians, may be subjected to changes in body temperature due to daily or seasonal changes in ambient temperature (Putnam & Bennett, 1981).

Early research into the effect of temperature on the locomotory capacity of amphibians showed that temperature had an almost instant effect on the jumping capabilities of frogs. Experiments on the effect of thermal acclimation on locomotion showed that thermal acclimation has little to no effect on locomotor performance (Putnam & Bennett, 1981). It was therefore concluded that locomotor performance in amphibians is thermally dependent and that most amphibians do not have the ability to thermally acclimate their locomotory performance to low temperatures (Bennett, 1990).

There are many studies that examine the ability of fish to acclimate their locomotory performance to different temperatures (Beddow & Johnston, 1995; Johnson *et al*, 1996). However, by comparison, studies of temperature acclimation of locomotory performance in amphibians, in particular, frogs, are lacking (Angilletta, 2009). The first study to show the ability of frogs to thermally acclimate locomotor performance to cool temperatures was a study conducted by Wilson & Franklin (1999). They showed that the maximum swim speed of tadpoles of the species *Limnodynastes peronii* was greater when tadpoles acclimated to 10°C were tested against tadpoles acclimated to 24°C in 10°C and 25°C water. Since then many studies have gone on to show that frogs do indeed have the ability to thermally acclimate locomotory performance (Wilson *et al*, 2000; McCann *et al*, 2014), while also reviewing the relationship between temperature and locomotion (Navas *et al*, 2008).

Despite the increase in scientific attention most studies have focused on a few species of *Rana* and *Bufo*, thus the ability to extrapolate from existing data and draw conclusions for other species is slightly diminished (Navas *et al*, 2008). This is especially prevalent for the species of *L. raniformis* and *L. ewingii* where research on the thermal effects on locomotion performance is lacking.



Locomotion plays an important role in migration, foraging and escaping predation (Bennett, 1990). *L. raniformis* and *L. ewingii* inhabit areas in the South Island of New Zealand that are subjected to large variations in temperature (Fig 3.1), thus the ability or inability to acclimate locomotory performance to temperature may restrict movement patterns and distribution limits (Wilson & Franklin, 2000). Therefore information on the physiological capabilities of these two exotic frogs is crucially important for understanding their distribution patterns as well as providing information that will aid in their conservation and protection in their native Australia.

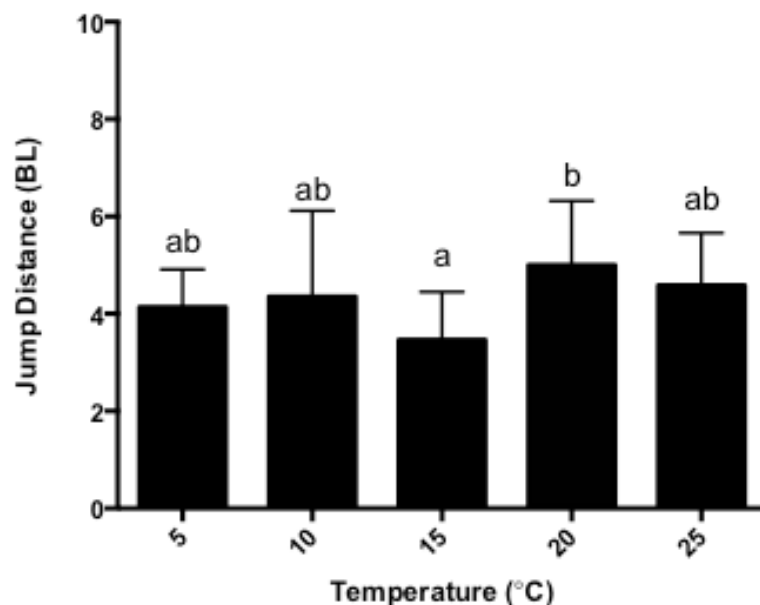
## **4.2 Methods:**

For methods used during the locomotion portion of this thesis refer to General Methods 2.1, 2.2 and 2.4.

After experimentation statistical analysis was carried out on the data using Excel and GraphPad Prism version 6. All jump distances were adjusted for body length of individual frogs. One-Way ANOVA was used in order to determine whether acclimation to a certain temperature had an effect on the amount of distance jumped at differing acute temperatures, while a Two-Way Repeated Measures ANOVA was done to compare the jump distance both intra- and interspecifically of both species after acclimation was completed.

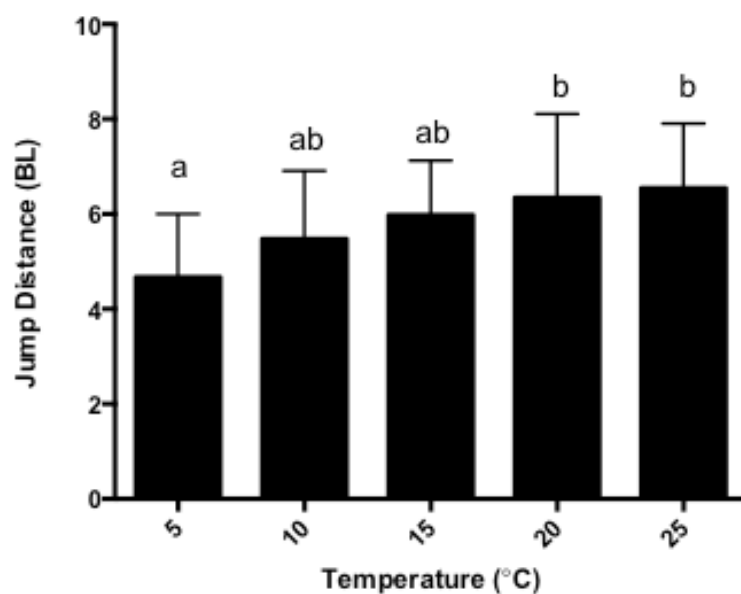
### 4.3 Results:

#### Locomotion: *L. raniformis*



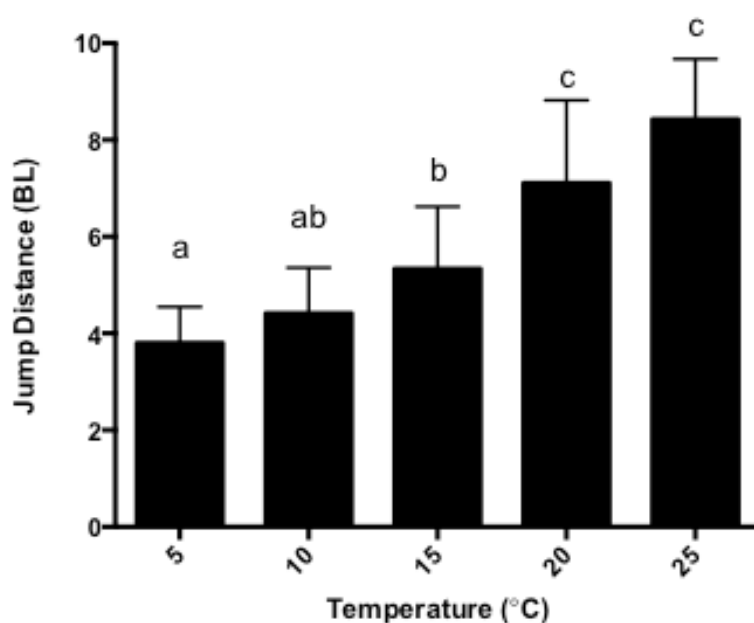
**Fig 4.1** Effect of temperature on the jump distance of *L. raniformis* after acclimation to 5°C. n=8. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

An ANOVA of the jump distance of *L. raniformis* after acclimation to 5°C showed that there was a significant difference in jump distance with relation to change in temperature ( $P = 0.0225$ ). A Tukey's test was done in order to determine which jump distances differed significantly from one another. The test showed that jump distance at 15°C and 20°C differed significantly, however, they did not differ significantly from 5°C, 10°C and 25°C (Fig 4.1).



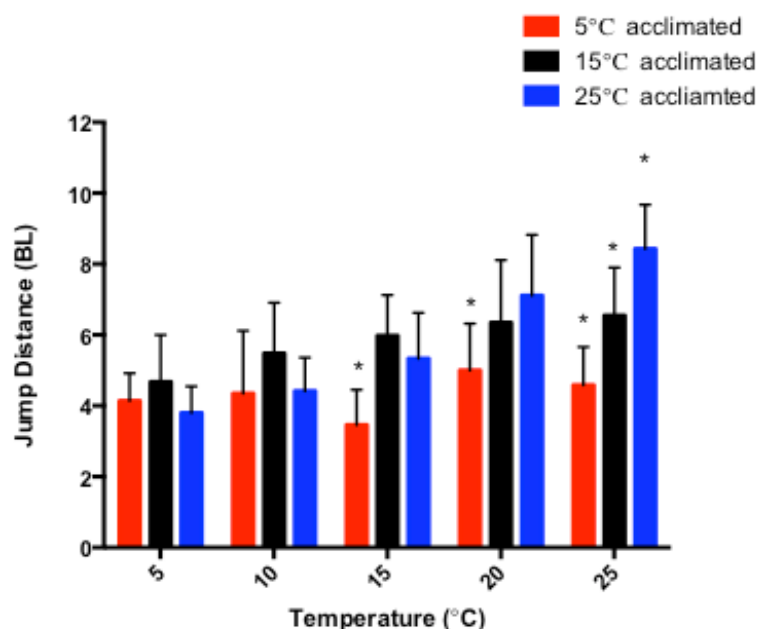
**Fig 4.2** Effect of temperature on the jump distance of *L. raniformis* after acclimation to 15°C. n=9. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

An ANOVA of the jump distance by the frog *L. raniformis* after acclimation to 15°C showed that there was a significant difference in jump distance when exposed to differing acute temperatures ( $P = 0.0018$ ). A post-hoc Tukey's test was then conducted in order to determine at which temperatures the jump distances differed from one another. The test showed that jump distances at 20°C and 25°C differed significantly from 5°C. There was no significant difference in jump distance between 5°C, 10°C and 15°C, while jump distances at 20°C and 25°C did not differ significantly from 10°C and 15°C (Fig 4.2).



**Fig 4.3** Effect of temperature on the jump distance of *L. raniformis* after acclimation to 25°C.  $n=8$ . Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

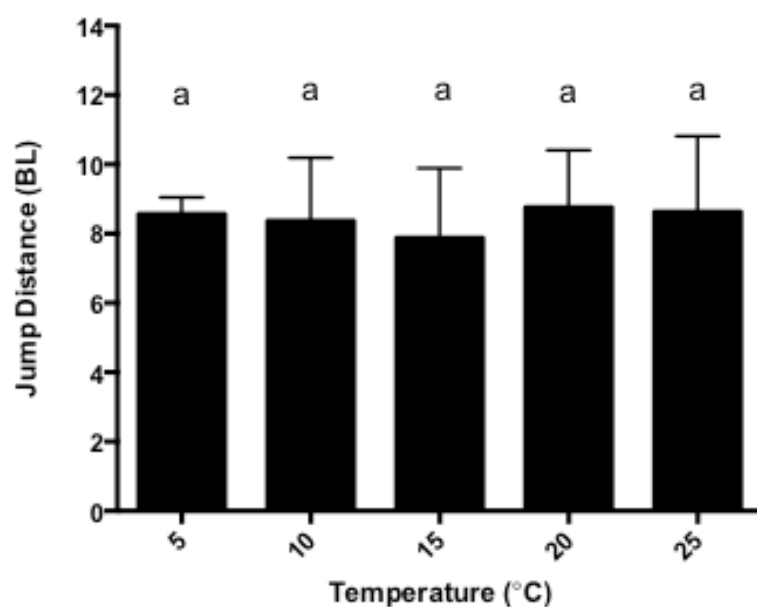
Analysis of the jump distances of *L. raniformis* after acclimation to 25°C showed that there was a significant difference in jump distance with relation to change in temperature ( $P < 0.0001$ ). A multiple comparisons test was run in order to show at which acute temperatures jump distance differed significantly. The test showed that at 5°C jump distance differed significantly from 15°C, 20°C and 25°C. Jump distance at 15°C differed significantly from 20°C and 25°C. However, jump distances at 5°C and 10°C, 10°C and 15°C, and 20°C and 25°C, respectively, did not differ significantly from one another (Fig 4.3).



**Fig 4.4** Comparison of the jump distances of *L. raniformis* after acclimation to three differing temperatures (5, 15 and 25). Error bars represent standard error. (\*) represent significant difference in jump distance ( $\alpha = 0.05$ ).

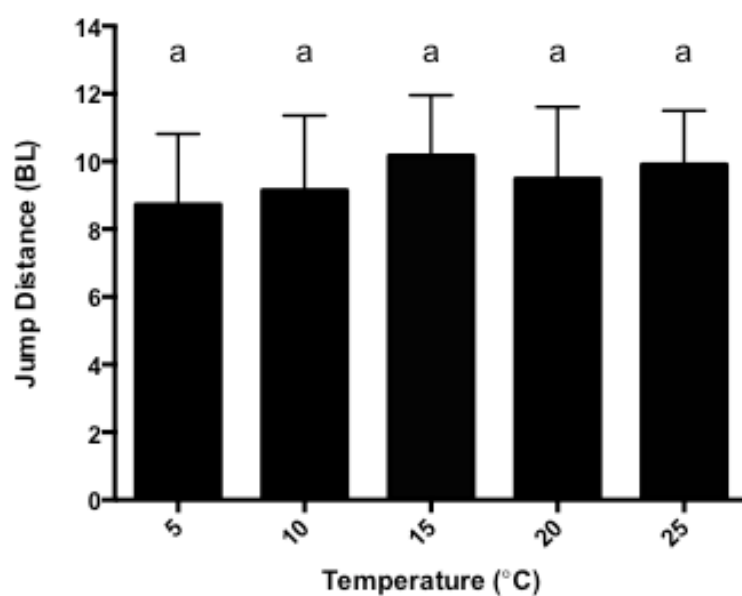
A Two-Way Repeated Measures ANOVA was carried out in order to determine how jump distance of *L. raniformis* differed at a range of temperatures after these frogs were acclimated for a month at three differing temperatures of 5°C, 15°C and 25°C. The results of the Repeated Measures ANOVA showed that there was a significant difference in jump distance and that acute and acclimated temperature, along with their interaction were significant sources of variation ( $P < 0.01$ ). A Tukey's multiple comparisons test was conducted in order to determine how jump distance of *L. raniformis* differed after acclimation for a month to set temperatures. The test showed that at 15°C jump distance of frogs acclimated to 5°C was significantly less than jump distances of frogs acclimated to 15°C and 25°C, however there was no significant difference in jump distance between the frogs acclimated to 15°C and 25°C. At the acute temperature of 20°C jump distance of the frogs acclimated for a month at 5°C was significantly less than the distance jumped by frogs acclimated to 25°C, however there was no significant difference in the jump distances of frogs acclimated at 5°C and 15°C or 15°C and 25°C. At the acute temperature of 25°C, jump distances at all three acclimated temperatures of 5°C, 15°C and 25°C were significantly different from one another. There was no significant difference in jump distance after acclimation to set temperatures of 5°C, 15°C and 25°C at the acute temperatures of 5°C and 10°C (Fig 4.4).

### Locomotion: *L. ewingii*



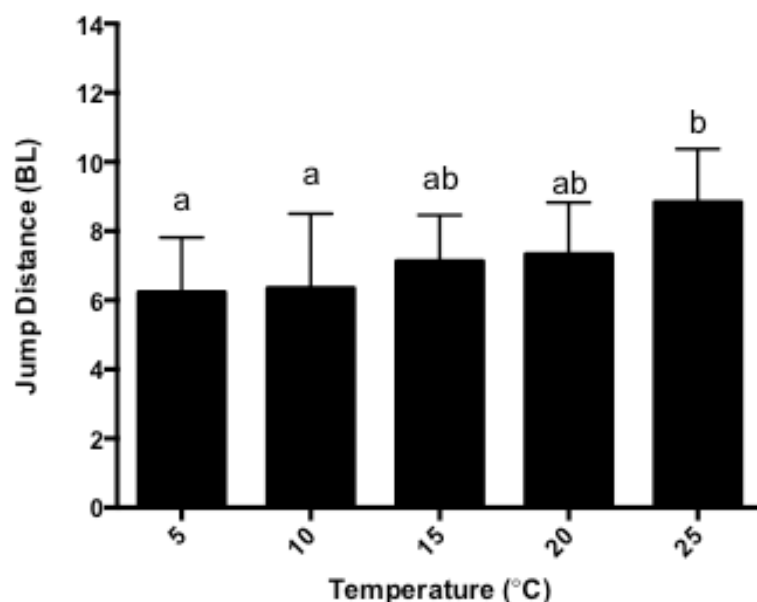
**Fig 4.5** Effect of temperature on the jump distance of *L. ewingii* after acclimation to 5°C. n=6. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

An ANOVA of the jump distance of *L. ewingii* after acclimation to 5°C for a month showed that there was no significant difference in jump distance with relation to temperature ( $P = 0.6907$ ). A multiple comparisons test also showed that there was no significant difference in jump distance between each of the acute temperatures following acclimation for a month at 5°C (Fig 4.5).



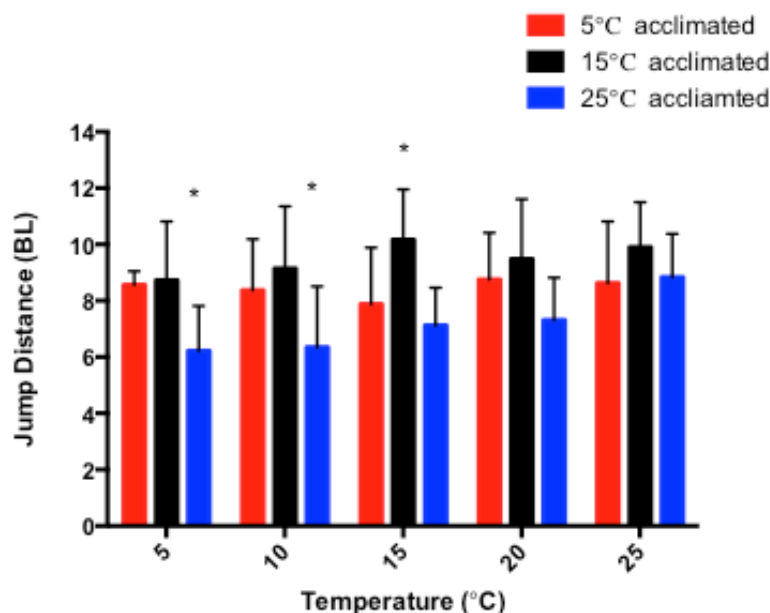
**Fig 4.6** Effect of temperature on the jump distance of *L. ewingii* after acclimation to 15°C. n=9. Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

Analysis of the jump distance of *L. ewingii* after acclimation to 15°C for a month showed that there was no significant difference in jump distance with relation to temperature ( $P = 0.2392$ ). A Tukey's test was done to see if any jump distances were significantly different from one another. The test showed that there was no significant difference in jump distance at acute temperatures after acclimation to 15°C for a month (Fig 4.6).



**Fig 4.7** Effect of temperature on the jump distance of *L. ewingii* after acclimation to 25°C.  $n=6$ . Error bars represent standard error. Temperatures with different letters are significantly different ( $\alpha = 0.05$ ). BL = Body Lengths.

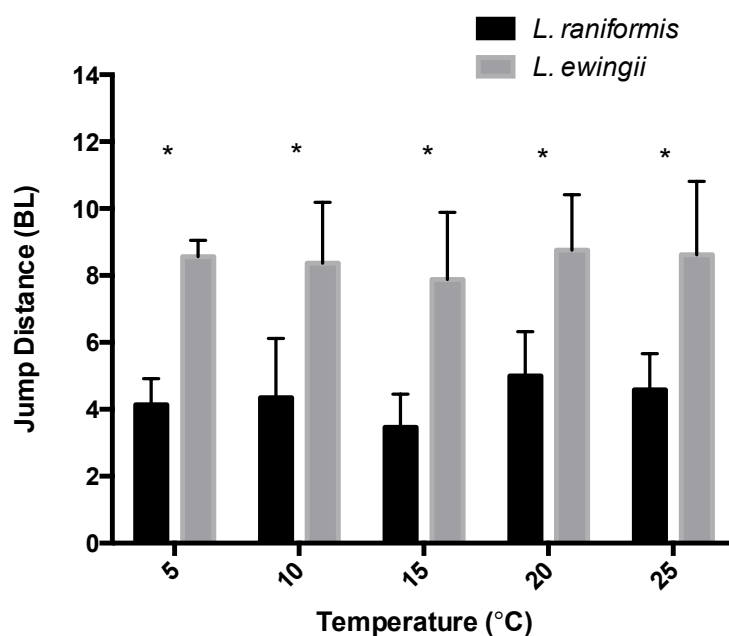
An ANOVA that was conducted showed that there was a significant difference in jump distance with relation to temperature of the frog *L. ewingii* after acclimation to 25°C for a month ( $P = 0.0177$ ). A post-hoc Tukey's test was then conducted to determine which jump distances were significantly different from each other. The test showed that at 25°C, jump distance was significantly higher than at 5°C and 10°C. There was no significant difference in jump distance between 5°C, 10°C, 15°C and 20°C, and 15°C, 20°C and 25°C, respectively (Fig 4.7).



**Fig 4.8** Comparison of the jump distances of *L. ewingii* after acclimation to three differing temperatures (5, 15 and 25). Error bars represent standard error. (\*) represent significant difference in jump distance ( $\alpha = 0.05$ ).

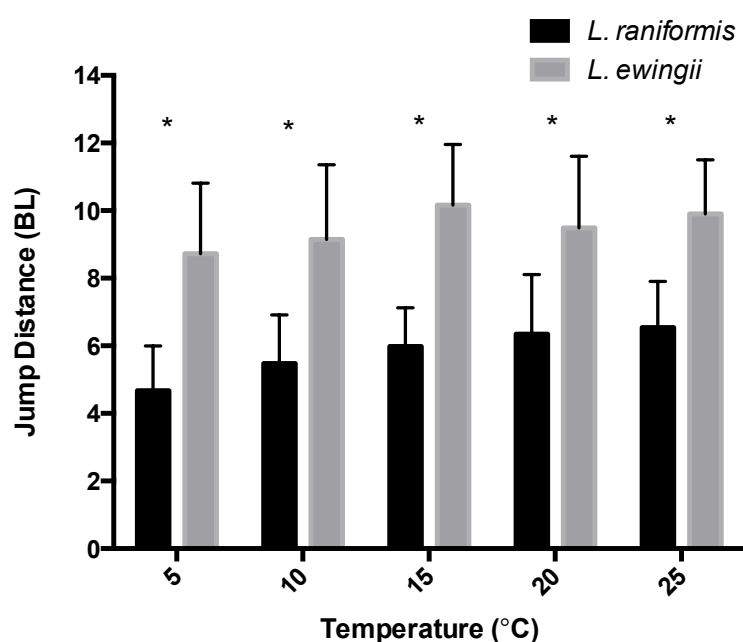
A Two-Way Repeated Measures ANOVA was carried out in order to determine how jump distance of *L. ewingii* differed at a range of temperatures after these frogs were acclimated for a month at three differing temperatures of 5°C, 15°C and 25°C. The results of the Two-Way Repeated Measures ANOVA showed that there was a significant difference in jump distance and that acute and acclimated temperatures were significant sources of variation ( $P < 0.025$ ), however the interaction between the two was non-significant ( $P = 0.1983$ ). A post-hoc Tukey's test was conducted in order to determine at which acute temperatures the jump distances of the frogs differed with relation to acclimated temperatures. The test showed that at 5°C there was a significant difference in jump distance between frogs acclimated to 25°C and 15°C, however there was no significant difference between frogs acclimated to 15°C and 5°C, as well as frogs acclimated to 5°C and 25°C. At the acute temperature of 10°C, there was a significant difference in jump distance for frogs acclimated to 25°C and 15°C, there was no significant difference in jump distance at 5°C and 15°C, and 5°C and 25°C after acclimation. For the acute temperature of 15°C the test showed that jump distance at 15°C was significantly different from 5°C and 25°C, however there was no significant difference in jump distance between 5°C and 25°C after acclimation. For the acute temperatures of 20 and 25°C the test showed that there was no significant difference in jump distance with relation to acclimation temperature (Fig 4.8).

### Locomotion: Comparison of *L. raniformis* and *L. ewingii*



**Fig 4.9.** Comparison of jump distance of *L. raniformis* and *L. ewingii* acclimated to 5°C for a month. Error bars represent standard error. (\*) represent significant difference of jump distance ( $\alpha = 0.05$ ). BL = Body Length

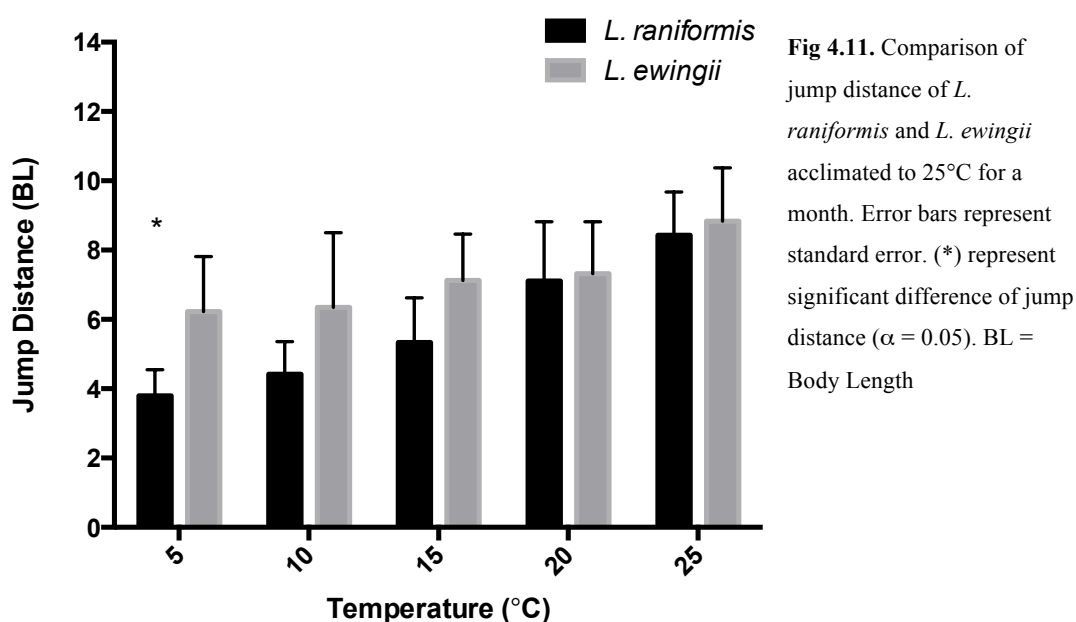
A Two-Way Repeated Measures ANOVA was conducted in order to determine if there were any significant differences in jump distance with relation to temperature between *L. raniformis* and *L. ewingii* after acclimation to 5°C for a month. The ANOVA showed that there was a significant difference and that acute temperature and acclimated temperature were significant sources of variation ( $P < 0.05$ ). A multiple comparisons test was conducted which showed that at all acute temperatures jump distance for *L. ewingii* was significantly higher than *L. raniformis* (Fig 4.9).



**Fig 4.10.** Comparison of jump distance of *L. raniformis* and *L. ewingii* acclimated to 15°C for a month. Error bars represent standard error. (\*) represent significant difference of jump distance ( $\alpha = 0.05$ ). BL = Body Length



Analysis of the jump distances of the two frogs *L. raniformis* and *L. ewingii* after acclimation to 15°C for a month showed that there was a significant difference in jump distance, with acute and acclimated temperature being significant sources of variation ( $P < 0.002$ ). A multiple comparisons test was conducted which showed that at all acute temperatures jump distance of *L. ewingii* was significantly higher than that of *L. raniformis* (Fig 4.10).



**Fig 4.11.** Comparison of jump distance of *L. raniformis* and *L. ewingii* acclimated to 25°C for a month. Error bars represent standard error. (\*) represent significant difference of jump distance ( $\alpha = 0.05$ ). BL = Body Length

Statistical testing of the results of jump distance of *L. raniformis* and *L. ewingii* after a month of acclimation at 25°C revealed that there was a significant difference in jump distance relating to temperature ( $P < 0.05$ ). A multiple comparisons test was then carried out in order to determine at which acute temperatures jump distance differed significantly between the two frog species. The test showed that at the acute temperature of 5°C there was a significant difference in jump distance, with *L. ewingii* jumping significantly further than *L. raniformis*. However, there was no significant difference in jump distances between the two species for the acute temperatures of 10°C, 15°C, 20°C, and 25°C (Fig 4.11).

#### 4.4 Discussion:

Locomotor performance in ectotherms is of particular importance as it not only allows escape from predators, but also aids in the ability of an animal to forage successfully, find a mate and move between suitable habitats when necessary (Wilson & Franklin, 1999). Due to the known effect temperature has on the physiological processes of amphibians it becomes important to be able to understand how temperature will affect processes such as respiration and locomotion. Without baseline data it becomes a lot harder to be able to make implications and draw conclusions; this becomes particularly prevalent when making decisions about invasive species control (McCann *et al*, 2014), or how to aid native species that have become endangered (Pyke, 2002). Moreover, this often leads to having to draw conclusions about a species based on work done on a similar or closely related species. This creates significant problems with anuran amphibians as the effect of temperature on behaviour and physiology is poorly known, and research is generally focused on a few species of *Rana* and *Bufo* (Navas *et al*, 2008).

In comparison to the frogs from the genus *Rana* and *Bufo*, the frogs from the genus *Litoria* have received far less attention, thus the ability to make implications and draw accurate conclusions is diminished. The purpose of this thesis is to provide baseline data on the physiological responses of these two frogs, *L. raniformis* and *L. ewingii*, to temperature. Current physiological information relating to these two frogs, especially from New Zealand, is lacking. This information will hopefully provide a baseline pool of data from which to draw from and as a result help in the understanding and conservation of these two frogs.

Early research into the effect of temperature on the locomotor performance of amphibians yielded mixed results. Work by Putnam & Bennett (1981) found that locomotor performance of the toad *Bufo americanus* was unchanged after acclimation to different temperatures, while Renaud & Stevens (1983) found that after acclimation to 25°C the frog, *Rana pipiens*, had an increased jump distance when compared to frogs acclimated to 5°C, however, when tested at 5°C and 15°C there was no difference in jump distance between the acclimated frogs; similar results have been observed in other amphibians, such as salamanders (Else & Bennett, 1987). Subsequently this led to a review that concluded that most amphibians do not have the

ability to thermally acclimate their locomotory performance to low temperatures (Bennett, 1990).

Work by Wilson & Franklin (1999) was the first to show that an amphibian, adult or juvenile, possesses the ability to thermally acclimate locomotor performance to cold temperatures. The frogs used in this experiment were the larval form of the frog *Limnodynastes peronii*. Subsequent work done by Wilson & Franklin (2000) found that the adult form of the frog *Limnodynastes peronii* did not possess the same ability to thermally acclimate locomotor performance to low temperatures. Similar work done on the frog *Limnodynastes tasmaniensis* found that after acclimation to either 9°C or 30°C for 60 hours jump distance did not significantly differ in the adult phase of this frog (Whitehead *et al*, 1989). It has, however, been pointed out that this acclimation time may not be sufficient enough to detect a change in jump distance due to acclimation. It has been suggested that early work into thermal acclimation did not account for confounding factors such as acclimation length, which may have caused thermal acclimation to go undetected (Feder *et al*, 1984).

Since these earlier studies, more attention has been given to the effect of temperature acclimation on the physiology of frogs. Recent work by McCann *et al* (2014) found that the cane toad, *Rhinella marina*, (used to belong to the genus *Bufo*) has reached areas of Australia that are much colder than the areas of its native habitat in tropical America. They found that these toads' critical thermal minimums were determined by exposure to cold temperatures over 12 hours. Thus these toads' ability to rapidly thermally acclimate is allowing for invasion of areas previously thought to be inaccessible. From the initial work on acclimation physiology more research has been done on the effects of acclimation. Rogers *et al* (2007) found that the frog, *Limnodynastes peronii*, shows sex specific thermal acclimation. They found that factors that affect the calling muscles were higher or had more activity than females after thermal acclimation; they also showed that males had a higher activity of metabolic enzymes in muscles using during amplexus (mating). Recent work on *Limnodynastes peronii* has shown temperatures experienced during embryonic development have an effect on thermal performance later in life. Seebacher & Grigaltchok (2014) showed that locomotor performance was greatest when acclimation temperature and embryonic development temperature were the same. However, from this research on *Limnodynastes peronii*, we can see the importance of

initial research and baseline data, as this allows further investigation that ultimately aids this frog and other amphibians in the long term.

Despite this recent attention to amphibians, in comparison to fish, data demonstrating the ability to thermally acclimate locomotor performance is lacking. Beddow & Johnston (1995) showed that in terms of burst swimming performance, the shorthorn sculpin, *Myoxocephalus scorpius*, possesses the ability to acclimate locomotor performance to differing temperatures. Similar examples can be seen in fish such as the goldfish and killifish (Johnson & Bennett, 1995). More recent work has shown that male mosquitofish, *Gambusia holbrooki*, after acclimation to 18°C and 30°C had greater sustained swimming performance when test temperature matched acclimation temperature (Hammill *et al*, 2004). Data on the thermal effects on locomotor performance of fish not only outnumbers data on amphibians, but is also more varied, in terms of species examined, and more detailed.

Data from this experiment on *L. raniformis* shows that after acclimation to 5°C there was no significant difference in jump distance aside from between 15°C and 20°C (Fig 4.1). This result is not unexpected as it has previously been shown that *L. raniformis* becomes torpid and unresponsive at low temperatures (Cree, 1984). After acclimation to 15°C there was a significant difference in jump distance at 20°C and 25°C when compared to 5°C (Fig 4.2). This indicates that these frogs are much more comfortable at higher temperatures closer to their acclimation temperatures and are therefore able to jump better. Acclimation to 25°C shows a standard Q10 response to an increase in temperature where jump distance increases as temperature increases. Jump distance was significantly higher at 20°C and 25°C compared to 5°C, 10°C and 15°C (Fig 4.3). This shows that frogs are much more capable at jumping at temperatures close to their acclimation temperature. When comparing the jump distances of the frog, *L. raniformis*, after acclimation to all three temperatures we can see that at the acute temperature of 25°C all three jump distances are significantly different from one another (Fig 4.4). This result shows that after acclimation for a month to 25°C, jump distance is significantly greater than jump distance when acclimation at 15°C and 5°C. This may indicate that *L. raniformis* possesses the ability to thermally acclimate to higher temperatures, but is not capable of acclimation to lower temperatures. This result mirrors what can be seen in the literature (Cree, 1984) and may also account for why *L. raniformis* is found predominantly in lowland temperate areas, compared the cooler, more alpine areas that *L. ewingii* inhabits.

Jump distance of *L. ewingii* after a month of acclimation to 5°C and 15°C showed that there was no significant difference in jump distance with relation to acute temperature exposure (Fig 4.5; 4.6). This result indicates that *L. ewingii* does not appear to acclimate locomotor performance to low temperatures, while after acclimation to 25°C we can see that jump distance of *L. ewingii* is significantly higher at the acute temperature of 25°C compared to 5°C and 10°C (Fig 4.7). However, when comparing all three acclimation temperatures together we can see that there is no significant difference in jump distance at 25°C after acclimation to all three acclimation temperatures (Fig 4.8). The data indicates that after acclimation to 25°C *L. ewingii* did not jump well at lower temperatures; this can be seen when comparing the data from the other two acclimation temperatures. This suggests that *L. ewingii* has acclimated to the high temperature, but in so doing has compromised jumping performance at lower temperature. Whether acclimation is always beneficial has been discussed in Angilletta (2009).

A comparison of the jump distance between the two species of *L. raniformis* and *L. ewingii* shows that after accounting for difference in body length between the two species, after acclimation for a month to 5°C and 15°C we can see that *L. ewingii* jumps significantly further at all acute temperatures, despite being a much smaller frog (Fig 4.9; 4.10). However, we can see that after acclimation to 25°C for a month that jump distance is significantly different only at the acute temperature of 5°C, with *L. ewingii* jumping further at that temperature (Fig 4.11). This result indicates that *L. raniformis* is a much more capable jumper at higher temperatures after acclimation to 25°C, compared to acclimation to lower temperatures, and also that despite acclimation to 25°C, *L. ewingii* is still a capable jumper at low temperatures, while *L. raniformis* is not. This result indicates that *L. ewingii* is capable of jumping regardless of acclimation temperature, while *L. raniformis* is only a capable jumper at higher temperatures after acclimation to high temperatures. This result may be due to *L. ewingii* being a much more terrestrial frog compared to *L. raniformis* (Cree, 1984), as well as being more tolerant of cold temperatures, exemplified by its ability to tolerate freezing (Bazin *et al*, 2007).

From these data we can begin to draw conclusions: the first being that *L. ewingii* is a better jumper than *L. raniformis*, this may be real or may be due to size difference, however the more terrestrial lifestyle of *L. ewingii* would lead to the assumption that it is the better jumper. The second conclusion is that with regard to

locomotion we have two very different animals. The data shows that *L. raniformis* is a warm habitat frog as it has its best performance at 25°C, and has issues jumping when acclimated to lower temperatures. By contrast, *L. ewingii* is a cold temperate frog and therefore does very well at low temperatures, but its performance is compromised at high temperatures.

## 5. General Discussion

### 5.1 Discussion:

Two frogs, *L. raniformis* and *L. ewingii*, were introduced to New Zealand over 100 years ago. Both frogs are native to Australia and are found in New South Wales, South Australia and Tasmania (Pyke, 2002). Since their introduction to New Zealand they have become well established and can be found throughout New Zealand (Fig 1.1). Though these frogs have been in New Zealand since the late 1800s they still exhibit similar biology to populations that remain in Australia (Voros *et al*, 2008). Despite the size difference between the two species (*L. raniformis*: 55-95mm, *L. ewingii*: 30-50mm) the frogs are relatively similar in their biology. One of the main differences between the two species is the habitat that they occur in with *L. raniformis* being found in much more aquatic environments, while *L. ewingii* can be found considerable distances from water in places such as grasslands and subalpine forests. Breeding biology is another difference between the species with *L. ewingii* breeding all year round, while *L. raniformis* breeds during the summer months (Cree, 1984; Pyke, 2002).

In their native Australia both frogs inhabit South-east Australia. The distribution of *L. ewingii* has remained stable, however, the distribution of *L. raniformis* has declined, with it once being widespread throughout South-east Australian it is now reduced to small populations within New South Wales, Victoria and Tasmania (Pyke 2002; Wassens 2008). Despite the declines experienced in Australia these frogs have become well established throughout New Zealand with *L. raniformis* found throughout New Zealand and *L. ewingii* being found primarily in the South Island with smaller populations in the North Island (Fig 1.1). From the populations in the South Island we can see that *L. raniformis* occurs primarily on the coast in more temperate areas, while *L. ewingii* occurs in much more alpine and cooler areas, however, there are areas in which their habitats do overlap and frogs from each species can be found in the other's habitat.

Amphibian populations are declining worldwide (Houlahan *et al*, 2000). One of the main causes of this decline is thought to be amphibian chytrid fungus (Skerratt, *et al*, 2007). This pathogenic, virulent and highly transmissible fungus is thought to be a primary cause for the extinctions and declines in amphibian population across the

world. The three *Litoria* species that are found in New Zealand (two of which are used in this study) have been shown to be susceptible to chytridiomycosis caused by the fungus, *Batrachochytrium dendrobatidis*, with the fungus being found in populations of these frogs in both the North and the South Island (Shaw *et al*, 2014). Anthropogenic influences have been cited as the other primary reason for amphibian declines across the world. These influences can range from human disturbance, which has been shown to alter the behaviour of juvenile frogs (Garner *et al*, 2008), to pesticides. Studies have shown that modified areas such as crop fields provide habitat for frogs, however, this also exposes the frogs to pesticides during critical stages of their life history, which can affect larval development and metamorphosis (Choung *et al*, 2011).

The IUCN has classified *L. ewingii* as ‘least concern’ with its population trend considered stable, while *L. raniformis* has been classified as ‘endangered’ with a decreasing population trend. Despite this, both populations in New Zealand remain stable (Newman *et al*, 2010). There has been an increase in ‘calls-to-action’ to help stop and prevent further population declines and extinctions in amphibians. These can range from scientific papers calling for further understanding on factors such as distribution and abundance (Pyke, 2002), or using places such as New Zealand for research on *Litoria* due to the stable populations that exist here (Bishop, 2008). New Zealand populations of *Litoria* frogs may also serve as an ‘Ark’ for declining Australian populations (Shaw *et al*, 2014). Getting the public involved and interested about amphibians is also important for their conservation. Many people already create protected environments for frogs in their gardens, as well as keeping exotic frogs as pets. Websites such as [www.nzfrogs.org](http://www.nzfrogs.org) provide valuable information and research on frogs in New Zealand. Childrens television shows such as ‘What Now’ aim to educate kids and raise awareness about frogs during NZ frog month by having people come on the show and talk to kids about frogs. All these aspects serve as important factors in helping to aid the conservation of frogs and amphibians locally and across the world.

All organisms possess some ability to change their behavioural or physiological characteristics in response to change in temperature. Prolonged exposure to certain temperatures can result in lasting changes in an animal’s thermal characteristics; this is often referred to as thermal acclimation (Angellitta, 2009). This discussion will focus on the effects of thermal acclimation on respiration and



locomotion of anurans as well as whether the effects of thermal acclimation on the physiology of these two exotic frogs, *L. raniformis* and *L. ewingii*, plays a factor in the differences seen in their distribution.

Early work on the effect of temperature on the respiration of toads showed that temperature acclimation had little effect on respiration rates (Carey, 1979). Subsequent work has shown that season may play an important role in thermal acclimation. Both the bullfrog, *Rana catesbeiana*, and the toad, *Bufo paracnemis*, have higher respiration rates when comparing summer and winter frogs across a range of temperatures (Rocha & Branco, 1998; Bicego-Nahas *et al*, 2001). Anurans from tropical and subtropical areas have been generally considered to lack the ability to thermally acclimate (Feder, 1982), however, it has been shown that the subtropical frog, *Rana latouchii*, has higher respiration rates when comparing summer frogs to frogs from other seasons across a range of temperatures (Chang & Hou, 2005). More recent work from Australia has shown that the tadpoles of the frog, *Limnodynastes peronii*, when raised in stable or variable thermal conditions do not possess the ability to thermally acclimate their respiration performance when tested over a range of temperatures (Niehaus *et al*, 2011).

Early work on the effects of thermal acclimation on locomotion physiology showed that some frogs possess the ability to partially acclimate their locomotor performance in response to thermal acclimation. Work by Renaud & Stevens (1983) showed that when the frog *Rana pipiens* was exposed to 25°C it jumped further at 20°C and 25°C compared to frogs exposed to 5°C. Conversely, they showed that the toad *Bufo americanus* jumped further at 5°C after being exposed to 5°C compared to toads exposed to 25°C. Locomotion experiments on the frog *Limnodynastes peronii* has shown that the tadpoles of this species possess the ability to thermally acclimate locomotor performance to cold temperatures, with tadpoles exposed to 10°C swimming faster than those exposed to 24°C across a range of temperatures (Wilson & Franklin, 1999), however the adults of this species do not possess the ability to thermally acclimate with frogs acclimated to 18°C not being able to jump when tested at 35°C (Wilson & Franklin, 2000).

Research on the thermal effects on the physiology of anuran amphibians faces two critical problems, the first being that despite the increase in academic attention as well as an increase in their public perception, the amount of physiological information on anuran amphibians is severely lacking, especially when compared with the

information available when examining the physiology of other vertebrates (Angilletta, 2009). This creates inherent problems when attempting to research certain species, as baseline data often doesn't exist for many species. Therefore a conceptual framework is often derived from other species or even other amphibians. The second critical problem is that despite the increase in research on anuran amphibians most of the research has focused on species from the genus of *Rana* and *Bufo* (Navas *et al*, 2008). This leads to inherent problems when comparing species as baseline data is lacking and thus leads to a lack of proper generalisations. A similar situation can be seen in the frog *Limnodynastes peronii*, where initial research has led on to more in-depth physiological research in this frog (Rogers *et al*, 2007; Seebacher & Grigaltchik, 2014). Whether this research is relevant to any other species aside from those in the genus *Limnodynastes* remains to be seen, however, it does highlight the importance that initial physiological research and baseline data can play in advancing the knowledge base of certain species, especially endangered or threatened species.

Information on both *L. raniformis* and *L. ewingii* is lacking. Most of the research that has been done on these two species involves their distribution patterns and life history traits (Pyke, 2002; Lauck *et al*, 2005; Wassens, 2008). In New Zealand the data that exists for these frogs is based almost solely on their distribution (Gill, 1973; Alderton, 1985; Voros *et al*, 2008). Physiological data on these two species is limited. Work by Cree (1984) showed that at 23°C respiration of both *L. raniformis* and *L. ewingii* were relatively similar, however, at 7°C *L. raniformis* became torpid and respiration was significantly lower than that of *L. ewingii*. This work also showed that larval survival was high for *L. raniformis* at 23°C, however mortality was high at 15°C, conversely, survival rates of tadpoles of *L. ewingii* was high at both 23°C and 15°C. Further work by Cree (1985) has shown that both *L. raniformis* and *L. ewingii* have the ability to rapidly rehydrate along with skin specialised for rapid water uptake. Both species are ammonotelic during the larval phase, while being strongly ureotelic during the adult phase. The more terrestrial *L. ewingii* has a lower rate of dehydration owing to the adoption of a water-conserving posture along with being less active, while despite being a semi-aquatic frog *L. raniformis* has some characteristics of a more terrestrial lifestyle. Out of the two species of frogs *L. ewingii* appears to be more tolerant of adverse conditions. Work by Cousineau (1990) found that *L. ewingii* bred all year round irrespective of temperature, rainfall or time of year. Tadpoles of this species were also observed in

acidic waters with a pH as low as 4.8 (Cousineau, 1990). *L. ewingii* has also been shown to be freezing tolerant, with up to 47% of its body water being frozen (Bazin *et al*, 2007). Subsequent work by Rexer-Huber *et al* (2011) showed that during freezing glycerol levels increased, then decreased upon thawing, while glucose levels remained the same during freezing but increased during thawing. This suggested that glycerol might be acting as a cryoprotectant. This work also showed that skin secretions from the frog *L. ewingii* contains active ice-nucleating agents, which aid in freezing survival by slowing the rate of ice growth and reducing the risk of injury.

When comparing the oxygen consumption of *L. raniformis* after acclimation to all three temperatures of 5°C, 15°C and 25°C we can see that the only significant difference seen in oxygen consumption is at 15°C for the frogs acclimated to 15°C for a month (Fig 3.5). This indicates that the frog *L. raniformis* was only capable of acclimating its respiration rate to 15°C. When comparing the oxygen consumption of *L. ewingii* after acclimation to all three temperatures we can see that oxygen consumption is highest when test temperature matches acclimation temperature (Fig 3.9). This indicates that *L. ewingii* is able to acclimate its respiration rate to high and low temperatures. A comparison of the two species shows that after acclimation to 5°C *L. ewingii* has significantly higher oxygen consumption compared to *L. raniformis* across all tested acute temperatures (Fig 3.10). This result is as expected, current literature indicates that *L. ewingii* is a much more cold tolerant frog and thus would be more capable of acclimating to cool temperatures, while *L. raniformis* has been shown to be much less active at cold temperatures (Cree, 1984; Bazin *et al*, 2007). After acclimation to 15°C for a month there is no significant difference in the oxygen consumption of both species when tested across a range of temperatures (Fig 3.11). This result is not unexpected as this temperature falls within the range that both species would be exposed to throughout the year (Fig 3.1). Acclimation to 25°C, however, shows a different story as at higher test temperatures *L. ewingii* has significantly higher oxygen consumption compared to *L. raniformis*. This result is unexpected as *L. raniformis* is generally found in warmer areas of New Zealand (Fig 1.1), thus is expected to perform better at higher temperatures. From this data we can see that *L. ewingii* possesses the ability to thermally acclimate its respiration rate allowing it to function optimally at a given temperature, while it seems that *L. raniformis* only seems capable of acclimating its respiration rate to higher temperatures.

When examining the data on the effect of acclimation on locomotion we can see that the data shows that *L. ewingii* is a cold temperate frog rather than a warmer habitat animal. This is exemplified by its ability to jump well at low temperatures (1.9 times further than *L. raniformis* at 5°C and 1.6 times further at 15°C) but not at high temperatures. Figs 4.7 and 4.8 show that at high temperature overall jump performance is compromised, so it is jumping a smaller distance at high temperatures compared to low temperatures, thus the question becomes whether *L. ewingii* does not show acclimation, or whether it has acclimated to a high temperature but in so doing is no longer a cold tolerant frog. By contrast the data shows that *L. raniformis* is an animal adapted to warm temperatures. Therefore acclimation to 5°C has clearly affected its physiology as it has a poor jumping performance irrespective of test temperature. This is important, as frogs do not show the expected Q10 effect. At the other end of the spectrum, acclimation to 25°C gives a classic picture of temperature effects. Indeed *L. raniformis*, tested at 25°C gives the best response with jump distance around 8 body lengths. In regards to locomotion we can see that we have two very different animals. *L. raniformis* has its best performance at 25°C, and has issues jumping when acclimated to lower temperatures. By contrast, *L. ewingii* does very well at low temperatures, but its performance is compromised at high temperatures. In this regard Fig 4.11 is interesting as it shows that the lack of differences between the two species is because after acclimation to 25°C, *L. raniformis* has its best jump performance, while *L. ewingii* is on its way down with a compromised performance.

## 5.2 Limitations

Originally each acclimation temperature was to have its own set of frogs that would be acclimated at that temperature, thus avoiding the need to use a repeated measures design. This would help to avoid the possible confounding factor of reversible acclimation (Angeilletta, 2009). Sex of each frog was not determined. As with both *L. raniformis* and *L. ewingii* the female of the species is larger than the male. Both species of frogs were measured and weighed with *L. raniformis* ranging from 69-78mm and *L. ewingii* ranging from 34-49mm. The size range for *L. raniformis* falls within the range of large males to small females, while the size range for *L. ewingii* falls across the size range for both males and females. Work has been done that shows

that thermal acclimation has sex specific effects on the frog *Limnodynastes peronii* (Rogers *et al*, 2007), thus possibly creating a confounding factor.

### 5.3 Future work:

The intent of this thesis is to provide a set of baseline data from which to build a solid array of physiological data of these two frogs. This will potentially aid, not only in the conservation of these two frogs but anuran amphibians in general. Future work could look to examine whether the results seen under experimental conditions will match those seen in wild populations from differing seasons. This will allow a comparison and will help provide justification as to whether results gathered in a laboratory are ecologically relevant. This experiment was conducted on the adult phase of these frogs, as such, physiological experiments on the effects of temperature acclimation on embryonic development and tadpole phases of these frogs would help shed light on when these thermal tolerances are developed and how developmental conditions may affect later life-history stages (Seebacher & Grigaltchik, 2014), furthermore if these frogs do acclimate, can this be seen at the molecular level? Future work could look to measure any possible difference in aerobic and anaerobic pathways. These frogs are sexually dimorphic with the females of both species being larger than the males. Future work may look into whether these frogs show sex specific thermal acclimation of their metabolic capacity and whether this potential acclimation confers any reproductive success (Rogers *et al*, 2007). The frog *L. ewingii* has been shown to be cold tolerant and is able to survive having almost half of its body water frozen for a period of time (Bazin *et al*, 2007). This frog is found predominantly in cooler areas of the South Island of New Zealand (Fig 1.1) where temperatures regularly fall below zero in winter; however, the frog *L. raniformis* can also be found in these areas. The over-wintering strategy of the frog *L. raniformis* is relatively unknown (Pyke, 2002), thus investigation into whether this frog possesses the same over-wintering strategy as *L. ewingii* or whether it ‘hibernates’ during winter will be of importance. It has been suggested that the frog *L. raniformis* will ‘aestivate’ during dry conditions by burrowing into mud, however there is no physiological evidence supporting this (Pyke, 2002), thus work into whether this species does aestivate and how it deals with potential problems such as hypoxia is relevant to its conservation.

#### 5.4 Conclusions:

From this thesis we can see how the physiology of two exotic frogs, *L. raniformis* and *L. ewingii* responds to thermal acclimation and temperature change. We see that *L. ewingii* is a cold tolerant frog that is capable of acclimating both its respiration and locomotion to a range of temperatures, although in undergoing acclimation to high temperatures it appears that *L. ewingii* performs poorly at low temperatures and thus is no longer a cold tolerant frog. Conversely, *L. raniformis* appears to be a much more warm temperate frog. This is exemplified by its ability to acclimate to and perform well at high temperatures but struggles at lower temperatures. From this data we can begin to see the importance of temperature physiology and see how much of an effect it has on these two particular organisms. The original hypothesis of this thesis was that temperature is the main factor determining the distribution of these two exotic species in the South Island of New Zealand. The data from this experiment supports this hypothesis in that temperature and thermal tolerance is likely playing a large role in determining the distribution of these two exotic frog species.

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